The effect of eyespot caused by Oculimacula yallundae and O. acuformis on yield of winter wheat

Thesis

THE EFFECT OF EYESPOT CAUSED BY *OCULIMACULA YALLUNDAE* AND *O. ACUFORMIS* ON YIELD OF WINTER WHEAT

Rumiana V. Ray BSc. (Honours)

A thesis submitted in partial fulfilment of the requirements of the Open University for the degree of Doctor of Philosophy

September 2004

Harper Adams University College in collaboration with Syngenta Ltd

DATE OF SUBMISSION 08 SEPTEMBER 2004
DATE OF AWARD 20 DECEMBER 2004

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

AKNOWLEDGEMENTS

I would like to thank my Director of Studies Dr. Simon Edwards and my Supervisors Dr. Geoff Bateman and Dr. Pete Jenkinson for all their help and encouragement throughout my study. In particular, I am grateful to Simon for all his support and help with all molecular aspects of the project, Geoff definitely helped me by improving my scientific writing and Pete was always there for long discussions, valuable suggestions, and constructive criticism. I would also like to thank Dr. Mitch Crook for all his help on the single tiller work and lodging. I would like to thank all of the CERC staff past and present– Charles, Paul, Alison, David, Kate, Jan, and Matt, for their patience with me and their help with the field operations. I am grateful to Flavio Rocci and Katia Pirgozlieva for their help with the processing of a large quantity of the samples.

I would like to thank Syngenta for sponsoring my work and in particular, Dr. Jim Beck for supplying the primers.

In addition, I would like to thank Stoyan, Vassil and Luda and the rest of my student colleagues for all their friendship and support.

I am especially grateful to all my family in England and Bulgaria but in particular to Sean and Alex who were very patient with me and extremely supportive throughout my studies.

ABSTRACT

Field experiments (2000-2002) were carried out to determine the effect of eyespot caused by *Oculimacula yallundae* and *O. acuformis* (formerly known as W-type and R-type, respectively) on yield or lodging resistance of winter wheat and to identify any differences in the development of the two pathogens throughout the season.

A field experiment on artificially inoculated wheat was designed to clarify the effects of the two *Oculimacula* spp. on plant characteristics associated with lodging resistance and on yield of individual shoots and whole crop. Overall yield of winter wheat in the absence of lodging was reduced by 11% and 6% by eyespot caused by *O. acuformis* and *O. yallundae* respectively. Both species reduced the stem safety factor associated with lodging resistance, indicating their ability to cause lodging in winter wheat by reducing stem-bending strength.

The ability of *O. acuformis* to cause significant yield loss was demonstrated in a series of fungicide efficacy experiments in early-drilled winter wheat. *Oculimacula acuformis* developed more and caused greater yield loss in the first winter wheat in which other stem-base pathogens were absent in the early growth stages of the crop. Fungicide mixtures containing cyprodinil were consistently more effective in controlling eyespot caused by *O. acuformis* in first, second and third winter wheat crops.

Positive relationships were observed between DNA concentrations of *O. acuformis*, *O. yallundae* and *M. nivale* throughout the growing season, indicating that these species co-existed within the crop. While the DNA of *M. nivale* consistently increased more up to GS 39 in different field experiments, DNA of *Oculimacula* spp. increased at different times in the growing season and these increases were sometimes associated with the relative quantities of DNA of *M. nivale* at the early growth stages.

TABLE OF CONTENTS

LIST OF FIGURES **PAGE NO.**

LIST OF TABLES **PAGE NO.**

LIST OF PUBLICATIONS

Ray, R. V., Edwards S. G. and Jenkinson, P. (2002). Chemical control of eyespot and other stem-base pathogens in an early drilled first winter wheat crop. **In** : Proceedings of Brighton Crop Protection Conference, British Crop Protection Council, Farnham, pp. 589-594.

Ray, R. V., Jenkinson, P. and Edwards, S. G. (2004). Effects of fungicides on eyespot, caused predominantly by *Oculimacula acuformis*, and yield of early-drilled winter wheat. *Crop Protection* **23**, 1199-1207.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Geographical distribution

Eyespot disease was first noted in France in 1912 (Fron, 1912), but records of unidentified foot-rot, causing lodging in cereal crops and showing other symptoms typical of eyespot were also made by McKinney in the USA in 1925. The causal organism at the time was not identified although several species were suggested. Sprague (1931) first identified the fungus causing this foot rot disease as *Cercosporella herpotrichoides* Fron after obtaining spores on artificial media and later inoculating wheat plants and re-isolating the fungus. The fungus also proved to be morphologically identical with the one previously isolated in France by Foëx and Rosella (1933) and called by the authors' "fungus x". In England the disease was first recorded by Glynne in 1936, and not long after in Scotland, Denis (1944) recorded the widespread incidence of *C. herpotrichoides* on cereal crops leading to extensive lodging and loss of crop of up to 50%. In addition, in a cereal survey in Ireland in 1955 McKay *et al.* (1956) reported severe outbreak of eyespot, caused by *C. herpotrichoides* on spring-sown oat crops badly affected by lodging. In Holland, Oort (1936) reported the widespread occurrence of eyespot disease on wheat and barley with more severe outbreaks on clay and sandy clay soils.

Eyespot occurrence has also been well documented in other parts of the world such as South Australia (Adam, 1940; Butler, 1957), New Zealand (Saxby, 1943), Russia, Greece, Poland (Anon, 1961), Japan, (Furuya, 1984) and Ukraine (Kryuchkova, 2000). Depending on the country origin several names have been used for eyespot disease, for example piètin-verse in France, strawbreaker or foot rot in the USA and eyespot in UK. Other terms describing lodging caused by the disease such as "straggling" have also been used widely by British farmers.

Symptoms

Sprague (1931) described the symptoms of eyespot as white, brown–borded elongate (rarely circular) spots up to 3 cm in length, occurring on outer leaf sheaths at the base of culms, later penetrating the stems causing the typical light coloured, later brown to black, charred–appearing lesions at ground level. Although eyespot lesions are generally confined to the lower part of the stems, in prolonged damp weather they may occur several centimetres higher (Fitt, 1988). Oort (1936) observed symptoms on wheat sown in early September or in October as small elliptical dark-bordered discolorations on coleoptile at the level of the soil. Later similar lesions were observed on the outer leaf sheaths with the fungus penetrating those in a radial way from the outer to the inner ones, producing black patches of mycelium, which may unite resembling symptoms of infection by *Ophiobolus graminis*. The development of black stromatic pustules within the centre of the lesion is commonly associated with eyespot infection (Ponchet, 1959, Booth & Waller, 1973, Goulds & Polley, 1990).

Symptoms of the disease are generally similar for wheat and barley, but they may be obscure on some cereals, such as oats. For example, although McKay *et al.* (1956) observed the occurrence of typical eyespot lesions to some extent on oat plants, they appeared rather scanty and frequently an indented line was present on one or both sides of the culm. The authors described this indentation as if the culm had been pressed in with the thumb–nail, the dent extending up the plant from the base to a height of 25 to 75mm, sometimes causing a split in the culm.

Severity of eyespot infection is dependent upon the growth stage of the crop and the rate of penetration of the disease. Infection can take place soon after seedling emergence, when decay of the basal parts of young leaves leads to premature yellowing of the blades (Fitt, 1988). Severe infection in early autumn sown crops can cause the death of

diseased shoots or even plants leading to thinning of the crop. Later in the crop development, if severe attacks of eyespot occur, the stems may become ridged, kinked, frayed, or otherwise distorted at the diseased zone, often filled with a grey fungal growth. The distinctive severe eyespot lesion may cause the straw to twist, bend, or even break at this point.

The ultimate effect of the fungus is to weaken the straws to an extent that they collapse in all directions (Butler, 1957). Random lodging ("straggling") of diseased crops occurs when individual stems collapse and are intermingled with upright and unaffected plants. Eyespot-induced lodging of crops can be distinguished from lodging caused by wind, rain, storm or over–use of nitrogen even if they all occur in the same crop. Crops lodged by the effects of natural forces usually fall in one direction with a gradual bending towards ground level, compared to eyespot diseased crops, which have fallen straws bent sharply and are characteristically twisted at/or just above ground level so that they lie closely pressed to the ground (Butler, 1957). Mature crops infected later in the season or where the disease is progressing slowly may fail to lodge but the ears produced may die prematurely.

Identification and diagnosis of eyespot can be difficult, particularly during early growth stages of the crop due to similarity of symptoms with sharp eyespot (*Rhizoctonia cerealis*) and brown foot rot (*Fusarium* spp. or *Microdochium nivale*) on the leaf sheaths and/or stems (Figure 1.1).

In mature plants eyespot may be distinguished from sharp eyespot, because sharp eyespot lesions have a dark edge border, the centres are much paler and usually rot, leaving a characteristic shredded area, and a "watermark" stain is often present on leaf sheaths above the lesions, which is never seen over eyespot lesions (Goulds & Polley, 1990). *Fusarium* spp. or *M. nivale* cause browning at the base of cereal shoots, however distinctive lesions are not apparent (Fitt, 1988; Goulds & Polley, 1990).

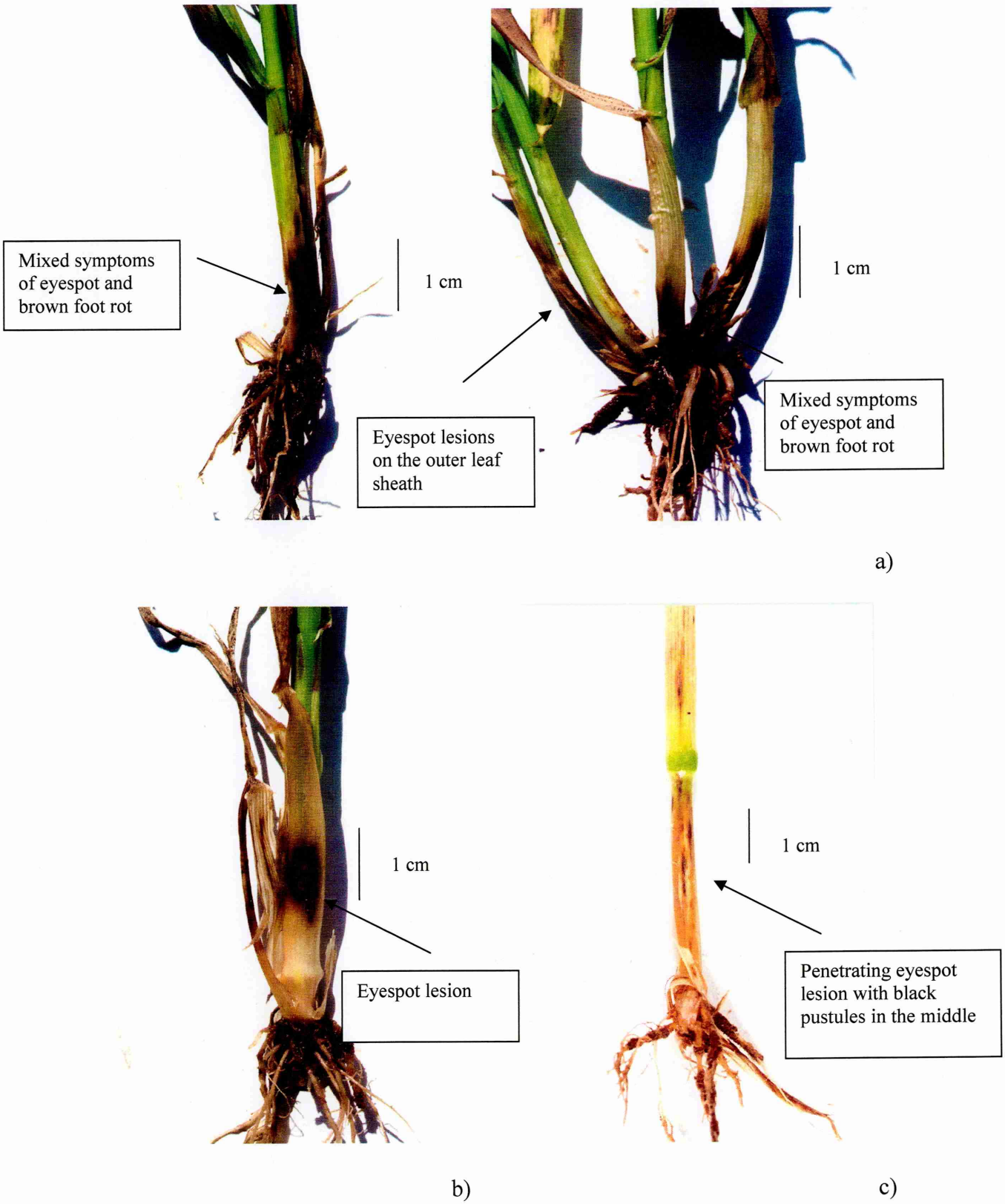


Figure 1.1. Young wheat plants at GS 30, showing mixed symptoms of eyespot and brown foot rot (a), eyespot only (b) and mature wheat plant at GS 69 with severe eyespot symptoms (c).

Shrivelled grain and partially empty, discoloured ears ("whiteheads") can often be found scattered throughout the crop (Saxby, 1943; Booth & Waller, 1973; Fitt, 1988). Consequently such ears are prone to secondary colonization by moulds, especially in favourable wet weather.

Causal organisms

Taxonomy

The fungus causing eyespot disease on cereals was first recognised as *Cercosporella herpotrichoides* Fron (Sprague, 1936). Deighton (1973) later re-allocated the fungus from *Cercosporella* to the genus *Pseudocercosporella* based on morphological differences. Nirenburg (1981) named two varieties of *P. herpotrichoides* and these were *P. herpotrichoides* var. *herpotrichoides* and *P. herpotrichoides* var. *acuformis* distinguished on the basis of conidial characteristics. Isolates of *P. herpotrichoides* var. *herpotrichoides* produced conidia which were usually curved, *P. herpotrichoides* var. *acuformis* produced usually straight conidia. Furthermore, the same author described two additional new species, *P. anguioides* and *P. aestiva*, also isolated from foot rot infected cereals and grasses. Von Arx (1983) re-classified *P. herpotrichoides* from *Pseudocercosporella* to the genus *Ramulispora* Miura that was restricted to graminicolous hosts. Von Arx (1983) defined *Ramulispora herpotrichoides* as having protuberent and multi-septate conidiogenous cells, acicular conidia with truncate, unthickened bases and frequent lateral branches.

The occurrence of the teleomorph of *P. herpotrichoides* described as *Tapesia yallundae* by Wallwork & Spooner (1988) further separated it from *Pseudocercosporella* since typically species from this genus have teleomorphs in *Mycosphaerella*. Furthermore,

Boerema *et al.* (1992) described *T. yallundae* var. *acuformis* as the teleomorph of *P. herpotrichoides* var. *acuformis* based on the distinct differences in the conidial anamorphs. Robbertse *et al.* (1995) later distinguished the two varieties as two separate species of *Ramulispora* based upon morphology, low percentage similarity in their DNA profiles and previous reports from other researchers regarding differences in infection process (Daniels *et al.*, 1991), distinct mating populations (Dyer *et al.*, 1994) and molecular markers (Julian & Lucas, 1990; Nicholson *et al.*, 1991, 1993; Priestley *et al.*, 1992; Thomas *et al.*, 1992; Nicholson & Rezanoor, 1994). The same authors also re-allocated *P. anguioides* to *Ramulispora* as *R. anguioides*, but failed to establish the genetic status of *P. aestiva* and retained the species in *Pseudocercosporella*. Stewart *et al.* (1999) carried out a phylogenetic analysis of rDNA sequences from 26 isolates of *Mycosphaerella* anamorphs and showed that all three species of *Ramulispora* formed one clade with *P. aestiva*, indicating affinities to *Tapesia* and not *Mycosphaerella*, thus *P. aestiva* was re-classified as *R. aestiva*. Based on phylogenetic analyses, Crous *et al.* (2000) confirmed that species of *Ramulispora* (*Tapesia*) grouped well outside *Mycosphaerella*. Following further phylogenetic analyses of the internal transcribed spacer (ITS)1, 5.8S and ITS2 rDNA sequence data of 39 isolates of *Mycosphaerellales* and *Helotiales*, Crous *et al.* (2003) recently revealed that the eyespot fungi associated with *Tapesia* are not congeneric with *R. sorghi*, the type of *Ramulispora* and that their anamorphs are distinct from *Mollisia* and *Tapesia* of the *Dermateaceae*. Therefore the authors proposed a new holomorph genus *Oculimacula* for the teleomorphs, *O. yallundae* and *O. acuformis* with respective anamorphs *Helgardia yallundae* and *H. acuformis*.

Classification and pathogenicity

Oculimacula yallundae (P. herpotrichoides var. *herpotrichoides)* and *O. acuformis (P. herpotrichoides* var. *acuformis)* were generally considered comparable with the two groups

of isolates of the fungus with different host specific pathogenicity W and R type, respectively (King & Griffin, 1985; Sanders *et al.*, 1986). R type isolates appeared to be equally pathogenic on wheat and rye seedlings whilst W type isolates were more pathogenic on wheat (Lange-de la Camp, 1966; Scott *et al.*, 1975). These pathotypes also differed in the rate of growth and morphology in culture, with the R type forming slow-growing colonies with feathery margins whereas W type had relatively fast growing colonies with even margins (Hollins *et al.*, 1985).

Extensive experimental work has been carried out in an attempt to define a clear system for identifying and classifying the isolates and/or pathotypes of the fungus. For example, Cavalier *et al.* (1987) used growth rate of isolates on potato dextrose agar (PDA) at 20°C to differentiate between slow growing and normal or fast-growing isolates of *O. acuformis* and *O. yallundae*, respectively. However, in many experiments differences in growth rate and/or colony morphology failed to correlate with pathogenicity types mainly because of cultural variation within the types (Creighton & Bateman, 1990; Thomas *et al.*, 1992). Alternatively, Creighton (1989) used colour production on maize agar to identify types, including intermediate isolates, which were not readily distinguished as W or R type on PDA. In some instances, pathogenicity tests to classify W and R type isolates also showed variable results. Fitt *et al.* (1987) tested 48 W and R type isolates of *Oculimacula* sp. for pathogenicity to wheat seedlings using mycelial or conidial inoculum and observed large variation in pathogenicity amongst individual isolates in both groups. Similarly, Creighton *et al.* (1989), after testing several isolates for pathogenicity on wheat and rye seedlings, noticed large differences in pathogenicity among isolates within each group. Both W and R type isolates were more pathogenic on wheat than on rye. The use of pathogenicity tests to distinguish between isolates of *Oculimacula* sp. was further complicated by the descriptions of new additional pathotypes, the C and S types. The two pathotypes C and S were differentiated on the basis of their pathogenicity to wild grass hosts, couch (*Elytrigia repens*) and goatgrass (*Aegilops squarrosa*), respectively

(Cunningham, 1981; Scot & Hollins, 1980). Nicholson *et al.* (1991) compared a range of techniques including colony morphology/growth rate, colour production on maize agar, pathogenicity on seedlings, spore morphology, and DNA markers. These researchers observed incomplete correlation between techniques especially on the basis of cultural characteristics/growth rate and spore morphology. DNA restriction fragment length polymorphisms (RFLP) revealed a higher degree of polymorphisms among the W type pathogenic isolates.

Julian & Lucas (1990) compared 101 isolates of *Oculimacula* sp. using isozyme markers and cultural characteristics. Isozyme polymorphism for esterase, glutamate dehydrogenase, malate dehydrogenase, glucose phosphate isomerase and malic enzyme separated W and R type isolates in agreement with pathogenicity tests. Additionally, isozyme markers also discriminated the two related species, *H. anguioides* and *H. aestiva.* Priestley *et al.* (1992) using isoenzyme profiling and DNA markers to identify pathotypes of several isolates of *Oculimacula* sp. confirmed the differences between W and R pathotypes and distinguished the C type isolates. Esterase binding patterns again clearly separated *Oculimacula* sp. from *H. anguioides* and *H. aestiva.* Nicholson *et al.* (1993) analysed 64 isolates of different geographical origin by RFLPs of mitochondrial (mt) DNA and ribosomal (r) DNA and concluded that the absence of intermediate mtDNA profiles among the two W and R types of *H. herpotrichoides* indicated that they may be genetically separated. Poupard *et al.* (1993) used the polymerase chain reaction (PCR) assay to amplify sequences of the ITS regions of rDNA genes from the different pathotypes in order to differentiate between them. Although a high percentage of homology within the same type was found, characteristic nucleotide differences (six nucleotide changes in the ITS 1 and three in the ITS 2 regions) allowed discrimination between the two types. Further evidence that the two pathotypes, W and R may be distinct species was provided by several studies based on molecular markers (Thomas *et al.*, 1992; Frei & Wenzel, 1993; Nicholson & Rezanoor, 1994; Takeuchi & Kuninaga, 1994; Takeuchi & Kuninaga, 1996, Gac *et al.*,

1996a). Simultaneously, more evidence emerged regarding the relatedness between isolates of W, R, S, and C pathotypes of *Oculimacula* sp. and *H. aestiva* and *H. anguioides*. For example, Nicholson & Rezanoor (1994), in a study based on random amplified polymorphic DNA (RAPD), investigating the variability between 23 isolates of W, R, C and S types from various locations, used 10-mer oligonucleotide primers to differentiate between the pathotypes. Cluster analysis of haplotypes based on all amplification products or only the major bands distinguished two main groups, one of which contained the R type isolates. The C type separated as a distinct group within the W/S type cluster. It should be noted that in this work the authors, however, used a limited number of isolates belonging to the C type (4) originating from the same geographical location. In addition, only one of these was tested for pathogenicity on couch, thus providing limited information on isolate variation within the type. However, in a later study Nicholson *et al.* (1995) demonstrated successful sexual mating between isolates of W, C and the S type indicating that these groups are closely related and remain discrete mainly on their capacity for pathogenic adaptation at the host-species level.

In the remaining part of this work, the current teleomorph names (*O. yallundae* or *O. acuformis*) of the two fungal species will be used throughout.

The discovery of apothecia of *O. yallundae* by Wallwork & Spooner (1988) further complicated the taxonomy of the eyespot pathogens. Since the first report of the sexual stage of the fungus in New Zealand (Sanderson & King, 1988), it has been found in many European countries (Hunter, 1989; Moreau *et al.*, 1989; King, 1990; Nicholson *et al*, 1991; Cavalier, 1994; Dyer & Lucas, 1995), South Africa (Robbertse *et al.*, 1994) and USA (Douhan *et al.*, 2002). Dyer *et al.* (1993) demonstrated in laboratory conditions that sexual reproduction was possible between complementary mating types, designated MAT-1 and MAT-2 of isolates of *O. yallundae* and the fungus exhibited two-allele heterothallic mating system. Although Moreau & Maraite (1996) confirmed these findings for *O. yallundae*, they observed that induction of sexual mating between compatible isolates of *O.*

acuformis was less successful, suggesting that this species may require specific environmental conditions to trigger the sexual cycle. The production and development of mature apothecia of *O. yallundae* in the field on stubble of wheat and barley crops and under controlled environment conditions have been reported on many occasions (Dyer *et al.*, 1994; Frei & Gindrat, 1995; Dyer *et al.*, 2001). In contrast, the sexual stage of *O. acuformis* has been shown to occur less frequently *in vivo* (Dyer *et al.*, 1994) and more complex to induce *in vitro* (Dyer *et al.*, 1996). For example, Dyer *et al.* (2001) developed a multiplex PCR based test to determine the mating type of isolates of *O. yallundae* and *O. acuformis*. Having analysed 62 field isolates from Europe and North America to determine the mating type distribution of *O. acuformis*, they observed that isolates of both mating types were present in both continents, indicating that a lack of compatible mating types is unlikely to be the reason for the rare detection of the sexual stage of the species. Furthermore, both Dyer *et al.* (1996) and Moreau & Maraite (1996) using molecular and isoenzyme markers demonstrated that *O. acuformis* also exhibited a two-allele heterothallic mating system, which is sexually incompatible with *O. yallundae* and thus a genetically separate species.

Epidemiology

Since the detection and the widespread occurrence of the sexual stage of the eyespot fungi in the field, necessary amendments were made to diagrams illustrating the life cycle of eyespot fungi (Figure 1.2) to include this significant change.

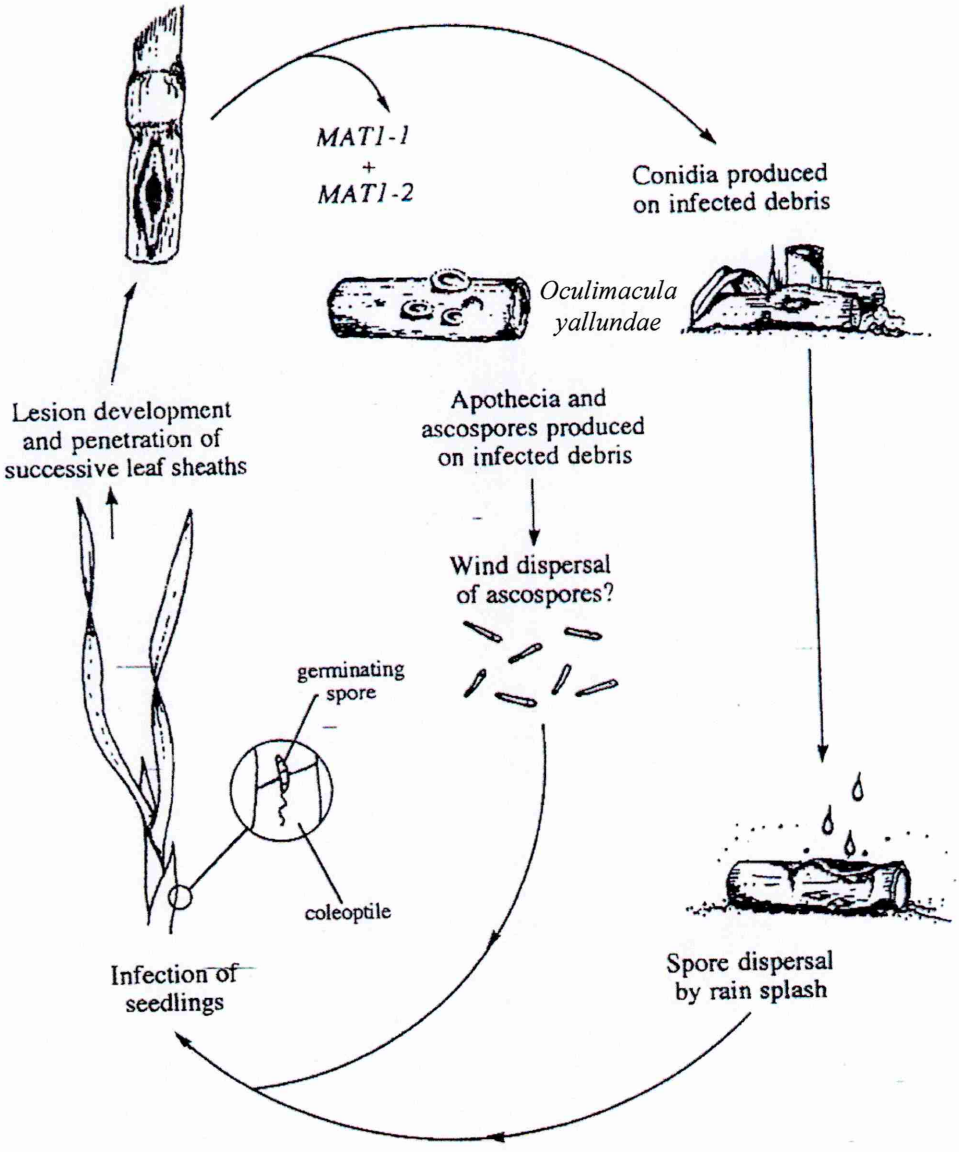


Figure 1.2. The life cycle of *Oculimacula* spp., showing asexual cycle with conidia and sexual cycle with apothecia and ascospores (Lucas *et al.*, 2000).

The importance of the sexual stage for the control of the disease is significant, since it can lead to greater variation within the pathogen population and novel genotypes as a result of sexual recombination, providing the pathogen with genetic flexibility to respond to selection pressures (Lucas *et al.*, 2000). Furthermore, ascospores can become airborne, providing long-range dispersal of inoculum capable of infecting cereal crops (Daniels *et al.*, 1995; Dyer & Lucas, 1995).

Survival and sources of inoculum

The eyespot pathogens can survive saprophytically on straw debris for a period of three years (Macer, 1961a). This may explain why researchers have found that breaks of two years have been insufficient to reduce the inoculum for effective control of the disease (Glynne & Moore, 1949; Cox & Cock, 1961; Macer, 1961a). Macer (1961b) demonstrated that the fungi exhibit a low competitive saprophytic ability and is therefore unlikely to survive in soil as a free-living saprophyte. Indeed, Blair (1954) failed to isolate the fungus from soil of diseased plants. However, Byther & Powelson (1966) observed that the fungi were able to germinate, grow and sporulate in field soil, incubated in controlled environment with temperature in the range of 5-25°C and concluded that the fungus was capable of short-term survival in soil. It is not clear, however, if these conclusions are valid for pathogen survival in natural field conditions.

The ability of the eyespot pathogens to compete with other saprophytes in the soil for the colonization of plant material added to the soil or preventing fungal invasion on straw have been shown to be weak (Bruehl & Lai, 1966; Garrett, 1975). Furthermore, Deacon (1973a) failed to notice any response to soil nitrogen level in longevity of survival of isolates of *Oculimacula* sp. although he observed large differences between isolates in their abilities to decompose cellulose and wheat straw, which however had no effect on

their survival period. The author also noted that the slow rate of straw decomposition was unable to support active, saprophytic survival and concluded that the fungus survived as resting hyphae.

Alternative hosts such as grass weeds may provide an important source of inoculum. Isolates of *Oculimacula* spp. have been recovered successfully from several grass species including annual meadow grass (*Poa annua*), couch (*Elytrigia repens*) and cocksfoot (*Dactylis glomerata*) (Lucas *et al.*, 2000). Apothecia development has been observed on great brome (*Bromus diandrus*) and barley grass (*Hordeum leporium*) straw (Wallwork, 1987). Recently, apothecia of *O. yallundae* have been detected on yorkshire fog (*Holcus lanatus*) in New Zealand (Dyer & Bradshaw, 2002). Nevertheless, the role of grass weeds in disease epidemiology remains unclear. Further studies are required to investigate the importance of grass weeds as a potential provider of ascospore or conidial inoculum for eyespot epidemics.

Production of inoculum

Eyespot inoculum may take the form of conidia or ascospores. The fungi sporulate to produce abundant conidia on infected crop debris (primary inoculum) remaining after harvest with a peak in sporulation in March/April, followed by a decline as the temperature increases, but with some spores still produced in June/July (Fitt *et al.*, 1988). Secondary inoculum produced on infected plant stems appears to play a less significant role in eyespot epidemiology (Rowe & Powelson, 1973a).

Laboratory experiments with naturally infected straw suggested that most asexual sporulation occurred between 1-20°C (Glynne, 1953; Jørgensen, 1964a). Further studies on both naturally infected debris and pure cultures have also indicated optimum temperature for conidium production between 5-16°C (Jørgensen, 1964a; Chang & Tyler,

1964; Drath & Rapilly, 1967; Rowe & Powelson, 1973b; Higgins & Fitt, 1984). Higgins & Fitt (1984) observed that on natural eyespot-infected wheat debris, sporulation decreased when the temperature was above 20°C, which stimulated the growth of competing fungi and bacteria. Rowe & Powelson (1973b) defined active sporulation periods for eyespot epidemics in which maximum sporulation can occur over periods of at least 2-3 weeks during which the humidity near the soil remained near saturation, the air temperature was above freezing for more than 8 h per day and the daily thermal sporulation coefficient (value based on the temperature–sporulation curve for *Oculimacula* sp.) was above 50. However as this method was developed using pure cultures, its relevance to naturally occurring eyespot epidemics is unclear. Further field studies investigating the applicability of this method under natural conditions would provide more information on its potential use as a forecasting tool for disease infection.

Another important factor for asexual sporulation of *Oculimacula* spp. is water. In many experiments sporulation only occurred on straw after water had been absorbed by it (Glynne, 1953; Rowe & Powelson, 1973b). Reports on the role of light for the production of spores appear conflicting. In some experiments light has had no effect on the ability of the fungus to sporulate (Glynne, 1953; Jørgensen, 1964a, Higgins, 1984) whereas in other experiments with pure cultures, incubated under near ultra violet light (NUV, blacklight) prolific sporulation has been observed (Chang & Tyler, 1964; Reinecke & Fokkema, 1979). Rowe & Powelson, (1973b) demonstrated that when infected straw was used as a substrate the fungus sporulated abundantly in darkness.

Ascospores represent an alternative source of air-borne inoculum, which could infect cereal crops for up to nine months of the year. Dyer *et al.* (1994) observed apothecia of *O. yallundae* on the stem bases of wheat straw stubble from mid-October to July, with peak numbers (up to 12 apothecia per stem) in late January to March. The development of apothecia was associated with mean monthly temperatures of 3 to 8°C. Rainfall appeared to be less important for the process. However, field surveys have shown that the incidence

of apothecia of *O. yallundae* is low. For example, Dyer & Lucas (1995) examined 45 set aside field sites from 1992 to 1994 in the UK and found apothecia on less than 3% of stubble stems. Apothecia of *O. acuformis* has been detected rarely (Dyer *et al.*, 1994; Dyer *et al.*, 2001) indicating that sexual reproduction of this pathogen is infrequent and is unlikely to be a significant inoculum source. It would also explain the lower degree of genetic variation detected in isolates of this pathogen (Nicholson & Rezanoor, 1994).

Dispersal of inoculum

The predominant inoculum in the field is in the form of conidia, which are dispersed from infected crop debris in rain-splashed droplets. Direct evidence of this process was offered by many experiments with simulated raindrops falling on infected debris or spore suspensions (Fatemi & Fitt, 1983; Fitt & Nijman, 1983; Fitt & Bainbridge, 1983; Soleimani *et al.*, 1996). In field studies, Fitt & Bainbridge (1983) failed to collect spores during dry periods or under light rainfall (<0.3 mm h^{-1}) but they collected large numbers of conidia during heavy rain (3-23 mm h^{-1}) after an initial period of wetting. *Oculimacula* spp. have also been shown to exhibit short range dispersal ability of 1-2 m from the inoculum source (Rowe & Powlson, 1973b; Fitt & Nijman, 1983) with most of spores carried in large rain droplets more than 400μm in diameter (Fatemi & Fitt, 1983; Fitt & Nijman, 1983). However, small numbers of air-borne spores (0.17-3.23 per m^{-3} of air sampled for 1.5-3h) have been collected over infected debris during rainfall (3 to 6 mm h^{-1}) (Fitt & Bainbridge, 1983) indicating the probability of dispersal by wind over long distances.

Infection

i. Factors affecting infection

On wheat seedlings, the coleoptile is the most susceptible tissue to infection by the fungus (Bateman & Taylor, 1976a & b). Since plants, remain susceptible to eyespot infection throughout the season and conditions favouring inoculum production remain favourable for long periods of time particularly in the UK where temperatures in winter/spring are usually within the range of 5°C to 16°C, environmental conditions during lesion development and establishment appear to be more important in determining the incidence and severity of the disease (Hollins & Scott, 1980; Fitt, 1988). Results from experiments with inoculated plants in controlled environment and glasshouse conditions have indicated temperature requirements of 5 to 18°C for eyespot lesion development (Defosse, 1966; Scott, 1971; Higgins & Fitt, 1985). In addition, it has been suggested that the growth of *Oculimacula* sp. in infected plants is a solely a function of temperature (Rapilly *et al.*, 1979). Indeed, both Defosse (1966) and Scott (1971) observed increased host penetration by the eyespot pathogen in response to an increase of the temperature and researchers have generally found significant relationships between accumulated temperature and disease progression in some seasons (Fitt, 1985). However, Higgins & Fitt (1985) reported that although the fungus penetrated leaf sheaths of inoculated wheat plants more rapidly at 10°C/15°C than at 5°C/10°C, death of leaf sheaths was also more rapid. Consequently there were fewer living infected leaf sheaths at 10°C/15°C than at 5°C/10°C and thus less fungal mycelium available for later stem penetration. Thus, the development of eyespot lesions and the penetration of successive leaf sheaths are also influenced by the production of new inner leaf sheaths by the plants and the death and eventual disintegration of outer leaf sheaths (Fitt, 1988).

Moisture is also required for infection to occur. Indeed most experimental inoculations with either mycelial or conidial inoculum have not been successful unless the site of infection was kept moist (Fitt *et al.*, 1988). Higgins (1984) reported that spore germination on potato dextrose agar (PDA) was sensitive to changes in water potential. Thus, at potentials below –4 MPa only 65% of spores germinated whilst at –2.7 MPa 95% of spores germinated. The optimum water potential for growth of the fungus on artificial media at 20°C has been reported to be in the range of –0.4 to –2.7 MPa (Bruehl & Manandhar, 1972; Higgins, 1984; Higgins & Fitt, 1985). Higgins & Fitt (1985) demonstrated that a heavy watering regime (plants were daily watered until free water was visible) or irrigation (equivalent of 23 mm of rain per day) for five weeks before harvest increased eyespot severity on winter wheat grown in soil columns by 40% and in the field by 25%. Rain also played an important role in providing humid conditions in the period of infection in addition to dispersing conidia. Van der Spec (1975) and Hollins & Scott (1980) reported a positive correlation between disease incidence and the number of wet days (4 days with more than 1mm of rainfall) during the period of exposure to inoculum. Defosse (1966) specified that 80-90% of relative humidity was necessary, irrespective of the ambient temperature for encouraging the infection process.

ii. The infection process

Dispersed conidia adapt the shape of the surface, sticking more readily to hydrophobic than hydrophilic surfaces on which they impact, wrapping around trichomes or following the topography of host epidermal cells. Daniels *et al.* (1991), using a combination of light and electron microscopy to study early eyespot infection of wheat seedlings, provided the first comparative report on the infection process for both *Oculimacula* spp. According to this report, the two species exhibit different patterns of coleoptile infection. Following spore adhesion and germination, *O. yallundae* produced orientated germ-tubes and penetrated

mainly through anticlinal cell walls, whereas *O. acuformis* invaded coleoptile tissues randomly. The penetration hyphae on *O. yallundae* entered the anticlinal cell walls and grew intramurally to the next cell layer where a network of branching hyphae colonised both anticlinal and periclinal walls (Lucas *et al.*, 2000). *Oculimacula acuformis* penetrated directly with very little intramural growth and a more prominent surface proliferation of mycelium, with the formation of runner hyphae, composed of several aggregated hyphae, encased in an extracellular mucilage sheath, promoting close adhesion to the host surface and possibly reducing desiccation (Daniels *et al.*, 1991). Both eyespot pathogens formed infection plaques on the longitudinal grooves overlying vascular traces of the leaf sheath. Infection plaques of the two species can be differentiated by their morphology, with *O. yallundae* forming loose, asymmetrical plaques and *O. acuformis* forming more compact plaques of closely associated cells (Daniels *et al.*, 1991). The main role of the infection plaques is the penetration of the host, the formation of adventitious hyphae, exploring the leaf sheath surface and initiating new foci of infection (Daniels *et al.*, 1991). Alternatively, this sclerenchyma–like tissue can survive on the surface of straw and is likely to be involved in the perennation of the pathogen (Lucas *et al.*, 2000). Deacon (1973b) observed morphologically similar, pigmented cells, produced in culture by *Oculimacula* spp., especially when nutrients such as nitrogen and dextrose were limited or at points of physical restriction. Lucas *et al.* (2000) suggested that physical cues may trigger plaque induction, and positioning of plaques over vascular tissues may be due to either topographical signals or differences in host exudates at these sites.

Leaf sheath penetration occurs by the formation of an infection hypha at the cell tip, puncturing the host cell wall. Simultaneous penetration by multiple cells located under the surface of an infection plaque gave the host cell wall a sieve-like appearance (Daniels *et al.*, 1991). Although the mechanism of penetration remains unclear, the localised erosion of the cuticle and alterations in the ultrastructure of the cell wall around the infection hypha implicated the action of hydrolytic enzymes (Lucas *et al.*, 2000). Mbwaga

et al. (1997) found higher activity of pectin methylesterase, polymethylgalacturonase, pectin lyase, carboxymethylcellulase, xylanase and arabanase in wheat plants inoculated with *O. yallundae* than in healthy control plants. However, since these enzymes were also found in non–inoculated plants the possibility that plant tissue could be stimulated by the infection to produce more enzymes cannot be overlooked. At a later stage of eyespot infection extensive tissue degradation develops. Coff *et al.* (1998) measured the quantity of the protein plastocyanin, essential for photosynthesis in eyespot diseased wheat plant tissue at GS 35 (Zadoks *et al.*, 1974), using enzyme linked immunosorbent assay (ELISA) and found that the protein was no longer synthesised, whilst in green healthy tissue it was still abundantly synthesised.

Infection caused by ascospores of *O. yallundae* has been shown to be similar to that by conidia (Daniels *et al.*, 1995). Bateman (Pers. Comm.) also achieved eyespot infection using ascospores of *O. yallundae* on wheat seedlings. In contrast, other researchers failed to attain infection of wheat or barley stems with ascospores of *O. yallundae* (Frei & Gindrat, 1995) and there are no reports on the infectivity of ascospores produced by *O. acuformis*.

iii. Comparative rates of infection and disease development of Oculimacula *spp.*

Several studies have been carried out on the comparative rates of infection and lesion development of eyespot when caused by *O. yallundae* or *O. acuformis*. *Oculimacula yallundae* has been shown to have faster penetration and stem colonisation rate in the early stages of disease development, expressed as higher severity score, compared to *O. acuformis* which progresses slowly early in the season but often at harvest, there are no differences in the severity of symptoms between the species (Goulds & Fitt, 1991a & b; Poupard & Cavalier, 1992; Poupard *et al.*, 1994). More recently, Nicholson *et al.* (2002) used quantitative PCR to follow the development of stem-base pathogens in winter wheat

and showed that, where *O. yallundae* was present in quantifiable amounts, symptoms of the disease developed earlier usually before GS 32. Goulds & Fitt (1990a) studied the early development of eyespot on field-grown wheat and barley seedlings inoculated with *O. yallundae* or *O. acuformis*. The authors reported that early differences between the species up to GS 31 were greatly influenced by crop and weather, and cold winters and winter wheat crop favoured the early development of *O. acuformis*. In contrast, a simultaneous study on winter wheat and barley in controlled environments by Bateman *et al.* (1990a), on the early infection by the two species showed no differential effect of temperature on the infection rate of the eyespot pathogens, indicating that other factors such as interactions between the stem base pathogens or host may be implicated in determining differences in the progress of disease when caused by the different *Oculimacula* spp. in the field. Bateman (1993) showed that under natural field conditions *O. acuformis* occurred more frequently than expected with *Fusarium* spp. and both eyespot fungi occurred frequently with *M. nivale*. Bateman & Munnery (1995) investigated further the influence of microbial interactions on differences in the rate of progress of eyespot disease when caused by each species in a controlled environment. They reported that *O. yallundae* was inhibited more than *O. acuformis* by *Fusarium* spp. particularly when the latter infected stems later then the former, suggesting that *O. acuformis* exhibited greater tolerance towards infections by secondary colonisers on the stem bases than O. *yallundae*, which may be a contributing factor in the increase of the population recovery of *O. acuformis* later in the season.

There is more recent evidence that *O. acuformis* is also capable of asymptomatic colonisation of wheat early in the season. Bateman (1993) isolated *O. acuformis* from stems exhibiting no visible symptoms more frequently than *O. yallundae*. Turner *et al.* (1999) showed that PCR assays could detect *O. acuformis* at GS 30 on plants where no eyespot lesions were observed. In such circumstances, decisions on eyespot control measures and timing would be impeded by the lack of visual symptoms.

The stem-base disease complex, interactions between diseases and their pathogens, and the use of visual identification and molecular diagnostics

Eyespot, sharp eyespot and brown foot rot comprise the stem base disease complex and often these three diseases occur simultaneously on the same stem bases. Brown foot rot is associated with many causal organisms, several *Fusarium* spp. and *Microdochium nivale* var. *majus* and var. *nivale*. However, in the UK, *M. nivale* was found to be the predominant pathogen isolated from brown foot rot lesions early in the season (Parry, 1990; Polley & Turner, 1995). Sharp eyespot is associated with a single causal organism – *Rhizoctonia cerealis*.

Symptoms of the stem base diseases can be indistinct and confounded, particularly at the early growth stages of cereals (Figure 1.1; Polley & Turner, 1995), which may lead to inappropriate or poorly timed disease control measures. Many researchers have successfully isolated both *Oculimacula* spp. from brown foot rot lesions (Brück & Schlösser, 1982; Bateman, 1993; Polley & Turner, 1995). However, *O. acuformis* has been found more commonly associated with the fungi causing brown foot rot (Bateman, 1993) indicating a higher degree of tolerance of secondary colonisers than *O. yallundae* (Bateman & Munnery, 1995). Turner *et al.* (1999), using quantitative PCR, found a significant positive association between *O. acuformis* and *M. nivale* var. *majus* on stem bases of winter wheat and between *Oculimacula* spp. and visual brown foot rot diagnosis or *M. nivale* var. *majus* and eyespot diagnosis. These researchers suggested that the two species *M. nivale* var. *majus* and *O. acuformis* are capable of coexisting in lesions of "indeterminate" type.

Interactions between *O. yallundae*, *O. acuformis* and *R. cerealis* have also been reported. Cavalier *et al.* (1987) reported that following mixed inoculation with both *Oculimacula* spp. on winter wheat grown in a glasshouse. *Oculimacula acuformis* reduced

the development of *O. yallundae*. However more recent statistical evidence from a long-term field winter wheat experiment for six years suggests that *O. acuformis* and *O. yallundae* do not interact (Bierman *et al.*, 2002). Natural antagonism between *Oculimacula* sp. and *R. cerealis* was reported by Brück & Schlösser (1982), who noticed that the two fungi were rarely present together in the same lesions on stem bases. Laboratory tests revealed that this apparent incompatibility was due to a production of diffusable, toxic substances by *Oculimacula* sp. and *Fusarium* spp., which inhibited mycelial growth of *R. cerealis*.

Fungicides can also affect the incidence and progression of the stem base diseases. In many experiments, researchers observed significant increases in the incidence of sharp eyespot (Prew & McIntosh, 1975; Reinecke *et al.*, 1979; Evans & Davies, 1986; Burnett, 1999) or brown foot rot (Reinecke *et al.*, 1979; Vilich-Meller, 1992), following fungicide applications for the control of eyespot.

Accuracy in the positive identification of the diseases early in the season is important because spray decisions for stem base disease control are made at GS 30/32 of cereals. In addition, investigation into the specific causal organisms is required for the effective control of the diseases, because of differences in fungicide resistance or sensitivity of the eyespot fungi (Bateman, 1990; Leroux & Gredt, 1997) and the fungi causing brown foot rot (Locke *et al.*, 1987). Traditional methods for species identification by plating infected, surface sterilized plant material on artificial media often underestimate the presence or relative amounts of pathogens on the stem bases because of different rates of growth of the pathogens concerned. For example, following isolation from mixed infection on winter wheat, *M. nivale* was out-grown by *F. culmorum* on PDA (Pettitt *et al.*, 1993). Similarly, using isolation from infected plant tissue on artificial media, the presence of *Oculimacula* spp. is often masked by other faster growing stem-base pathogens or saprophytes (Priestley & Dewey, 1993; Gac *et al.*, 1999). Molecular methods, used to detect and quantify stem base pathogens *in planta*, however, provide much more detailed

and specific information regarding amounts of pathogens present in disease complexes and single fungal infections.

Several PCR assays have been developed for the detection (Gac *et al.*, 1996b; Beck *et al.*, 1996) and quantification (Nicholson *et al.*, 1997) of *O. acuformis* and *O. yallundae*. PCR assays have also been developed to positively identify and quantify the varieties of *M. nivale* (Nicholson *et al.*, 1996) and *R. cerealis* (Nicholson & Parry, 1996). Gac *et al.* (1999) and Turner *et al.* (1999) used different PCR assays to evaluate visual assessment of eyespot when caused by *Oculimacula* spp. The findings of both works were similar. PCR assays detected *O. acuformis* pre-symptomatically and the visual disease caused by this pathogen developed more clearly later in season. In addition, Turner *et al.* (1999) found that visual assessment of individual diseases correlated poorly with PCR data where brown foot rot caused by *M. nivale,* and eyespot caused by *O. acuformis,* were present together up to GS30. Turner *et al.* (2001), in a further study of the relationships between visual assessment of eyespot incidence and severity and pathogen DNA quantified using PCR assays, determined that at the early growth stages, when spray decisions are made molecular assessments provided more reliable estimate of the abundance of the pathogens than visual assessments. Nevertheless, the lack of correlation between the early amounts of pathogen and disease severity later in the growing season presented an obstacle in making decisions on fungicide treatment based on early diagnosis and quantification.

Changes in the eyespot pathogen population

During the 1980s in the UK, the population in the eyespot fungi changed from being predominantly *O. yallundae* to *O. acuformis* (King & Griffin, 1985). Recent publications

suggest that, at present, *O. acuformis* is still the predominant pathogen causing eyespot disease in the UK (Birchmore & Russell, 1990; West *et al.*, 1998; Bardsley *et al.*, 1998). Fungicides, crop sequences and husbandry have all been implicated in the selection of *O. acuformis* in the UK eyespot pathogen population. King & Griffin (1985) first suggested the involvement of methyl benzimidazole carbamate (MBC) fungicides in the changes in the eyespot population. However, further experimentation using a variety of fungicide treatments in winter wheat has indicated that, although MBC fungicides selected resistant isolates more frequently in the population of *O. acuformis* than *O. yallundae*, prochloraz applications resulted in greater proportions of *O. acuformis* being recovered (Hoare *et al.*, 1986). The role of prochloraz in selecting for *O. acuformis* in the eyespot pathogen population is well documented (Bateman *et al.*, 1990b; Bateman & Fitt, 1991; Bateman *et al.*, 1995; Bierman *et al.*, 2002). The lower efficacy of prochloraz against *O. acuformis* was later explained with the discovery that isolates of this species have a wider range of sensitivity to prochloraz than *O. yallundae* in the UK, allowing selection of the less sensitive strains (Bateman, 2002). There are also resistant isolates of both *Oculimacula* spp. to prochloraz in France (Leroux *et al.*, 2003).

The increased cultivation of barley has also been suggested as a contributing factor to the increase of *O. acuformis* in the eyespot population. It certainly fitted well with studies on the pathogenicity of both species, which showed that *O. acuformis* is relatively more pathogenic on barley than on wheat compared to *O. yallundae* (Scott & Hollins, 1982). Bateman & Gutteridge (1996) recorded the incidence of eyespot fungi in sequences of wheat, barley and triticale and reported greater proportion of isolates of *O. acuformis* being recovered in three successive years from barley, and usually from triticale, than from wheat.

Early sowing of winter crops has also been implicated as a possible factor influencing changes in the eyespot pathogen population. For example, earlier sowing has

been suggested to favour more *O. acuformis* by allowing it a longer period to develop into a severe epidemic (Bateman & Jenkyn, 2000).

Control

In order to minimise the risk of severe eyespot occurring, several methods of control may be employed, including the use of cultural control techniques, the growing of resistant cultivars and the use of fungicides or biological control agents.

Cultural control techniques

Cultural control techniques including suitable crop rotations, sowing date, tillage techniques and use of fertilizers, may all contribute to the reduction of pathogen inoculum available for infection and spreading of eyespot disease within the crop. Some of the cultural control methods may, however, have differential effects on the stem-base diseases. For example, while ploughing could reduce the risk of brown foot rot, on some occasions it may encourage the development of eyespot (Prew *et al.*, 1995).

i. Crop rotation

Sprague & Fellows (1934) were among the first researchers to propose the inclusion of legumes, such as field beans, clover and lucerne in cereal rotations in order to control eyespot disease. Another crop in intensive cereal production considered as a good break crop for eyespot control was oats (Oort, 1936). However, following a cereal crop survey in

Ireland, McKay *et al.* (1956) reported a severe outbreaks of eyespot on oats, leading to more than 80% infection and lodging of the crop. Glynne (1942) carried out three 4-year surveys on arable crops to establish the effects of previous crops on eyespot incidence in cereals and concluded that continuous wheat or wheat and barley, grown in short sequences, were significantly increasing the risk of severe eyespot disease. The importance of the previous host crop for eyespot infection was indicated by many investigations (Defosse & Rixhon, 1968; Cook *et al.*, 1991). Slopek & Labun (1990) carried out a survey of more than 50 barley fields for eyespot incidence and observed the highest disease incidence following barley, wheat, oats or fallow and lowest following canola, sugar beet or grass. Similarly, Vilich (1993) investigated the effect of rotations of mixed and pure stands of winter wheat and barley on stem base diseases for a three year period (1989 to 1991) and observed that eyespot incidence in barley or wheat increased significantly more when grown after barley than it did after mixtures or after wheat.

Previous crop may affect the two eyespot pathogens differently as demonstrated by Bateman & Gutteridge (1996). These researchers monitored the incidence of eyespot and population structure in consecutive crops of winter wheat, barley and triticale and determined that barley and triticale selected for eyespot populations with a greater proportion of the *O. acuformis* (average for both 87%) than wheat (50%). This confirmed earlier reports by King & Griffin (1985) that, in England and Wales three or more consecutive barley crops increased the proportion of *O. acuformis* isolates in a subsequent cereal, while wheat crops had the opposite effect. More recently, during a two-year survey (1997 to 1998), Bardsley *et al.* (1998) assessed more than 170 samples for incidence and severity of stem base diseases in the UK and reported that more than 62% of samples taken following a broad leaf crop were infected with *O. acuformis,* indicating that a one year break crop had little effect on reducing eyespot inoculum when caused by this species.

Colbach *et al.* (1997) suggested that, after environment, the most important factor in determining disease risk was the interaction of crop succession and tillage, as it

influenced the amount of infectious crop residues close to the soil surface. In a second study, Colbach *et al.* (1999) proposed a dynamic model of the effect of rotation and crop management, on the frequency of eyespot infected plants. The model incorporated two parameters, associated with the primary (from infectious crop residues) and secondary (from living diseased plants) infection cycles. The authors suggested that the primary infection cycle depended on micro-environment, crop rotation, soil tillage, sowing date, tiller number per plant and available nitrogen, whilst the secondary infection cycle depended on tiller number per plant. Although the model permitted ranking of the factors influencing eyespot development, it failed to identify the factors determining the micro-environment, for example climatic conditions or soil type.

Eyespot pathogens have been shown to survive successfully on volunteers and weeds on a set–aside with natural regeneration for 3-5 years and still cause significant disease on the following winter wheat (Smith *et al.*, 2000).

ii. Land preparation

Both pathogens causing eyespot are capable of surviving saprophytically on crop debris (Jalaluddin & Jenkin, 1996). Therefore it is expected that the removal or burial of crop debris by ploughing-in would significantly reduce the risk of eyespot infection and disease. The evidence of much research however suggested that in many cases eyespot was more severe after ploughing than after non-inversion tillage (Mielke, 1983; Murray *et al.*, 1991; Prew *et al.*, 1995). Colbach & Maynard (1995) observed that the amount of infectious residue on the soil surface depended not only on soil tillage but also on crop succession. The authors showed that where the previous crop was a host crop, preceded by a non-host crop, soil inversion buried host residue, thus decreasing the infection risk. Where the previous crop was a non-host preceded by a host crop the opposite effect was observed as ploughing carried the host residues back to the soil surface. According to Macer (1961a),

stem bases previously infected by eyespot could remain effective sources of inoculum for up to 3 years when buried.

Straw management has been shown to have a limited effect on the reduction of eyespot inoculum. Colbach & Saur (1998) reported no effect on eyespot severity and infection with either straw burial or removal. Prew *et al.* (1995) observed more severe eyespot and sharp eyespot after straw was burnt than following straw incorporation. Smiley *et al.* (1996) confirmed that burning increased eyespot incidence especially in high fertility soils. Jalaluddin & Jenkin (1996) reported less sporulation of *Oculimacula* spp. in the presence of chopped wheat straw than when the straw was absent. It is possible that straw may sometimes interfere either with production or dispersal of conidial inoculum or with infection (Bateman & Jenkin, 2000). For example, suppression of Fusarium foot rot in glasshouse experiments and of eyespot in the field by straw of oilseed rape or field beans has been observed (Bateman & Jenkin, 2000).

iii. Sowing date and rate

Early sowing has been reported to favour eyespot (Oort, 1936; Bruehl *et al.*, 1968; Cook *et al.*, 1991; Colbach & Saur, 1998) mainly because it extends the period for infection and development of the disease. In addition, a high sowing rate (300 kg ha^{-1}, Higgins *et al.*, 1986) and row spacing have been reported to influence the severity of eyespot indirectly by increasing the canopy density and humidity at the soil surface, thus creating conditions more favourable for the infection and spread of the disease (Bruehl *et al.*, 1968). Covarelli & Santori (2000) investigated for two years (1998 to 2000), the effect of three sowing dates and fungicides on the incidence of *Oculimacula* sp. in winter wheat and reported that delayed sowing showed more than 80% reduction in eyespot incidence. In a survey of winter wheat crops carried out by West *et al.* (1998), a greater proportion of *O. acuformis* (86%) was found in the earlier drilled crops (before 15 September). Bardsley *et al.* (1998)

reported that later drilled crops infected with *O. acuformis* had less severe eyespot than those drilled earlier. Bateman & Jenkyn (2000) suggested that these differentiating effects of sowing date were based on differences in rates of disease development of the species rather than differences in their survival abilities.

iv. Nitrogen inputs

The reports on the effects of nitrogen on eyespot disease appear contradictory, which may be because of the use of different forms of nitrogen and rates, different cultivars, differences between the two pathogen species, inoculum levels or environmental factors. Early glasshouse experiments carried out by Glynne *et al.* (1945) using inoculated wheat plants receiving different rates of nitrogen demonstrated that eyespot reduced yield of plants with high nitrogen (375 kg ha^{-1} of ammonium sulphate) by 16% compared to 44% with low nitrogen rates (112 kg ha^{-1} of ammonium sulphate). The researchers observed the production of larger number of tillers when nitrogen was applied, enabling the less severely diseased shoots to survive and produce ears. In contrast, Bruehl *et al.* (1968) carried out eyespot inoculated field trials with winter wheat and observed that nil, moderate (89 kg ha^{-1} of anhydrous ammonia) or high (179 kg ha^{-1} of anhydrous ammonia) rates of nitrogen, applied before sowing had no effect on disease causing yield losses of 8, 12 and 10% respectively. Smith *et al.* (2000) also failed to observe any effect on eyespot disease following applications of nitrogen applied as ammonium nitrate (95 to 225 kg ha^{-1}) on two successive winter wheat crops sown following set–aside for 5 years. Cunningham (1966) showed that nitrogen in the form of calcium ammonium nitrate at 375 kg ha^{-1} applied at sowing of spring cereals increased percentage of eyespot diseased wheat straws by 26 %. This increase in eyespot was associated with indirect effects of nitrogen fertilisation such as early tillering and increased weed growth providing the high humidity conditions required for sporulation and secondary infection by the pathogen. Smiley *et al.*

(1996) observed that eyespot in winter wheat increased with the rate of applied nitrogen (45 to 180 kg ha^{-1}) and was inversely proportional to soil pH.

It is possible that the form of nitrogen applied may have affected disease development. For example, Colbach & Saur (1998) reported that wheat in early sown plots fertilised with ammonium sulphate had less eyespot than wheat fertilised with ammonium nitrate. This however, contradicted a previous report by Lucas *et al.* (1988) that eyespot was more severe in wheat plots fertilised with ammonium sulphate and less in ammonium-nitrate fertilised plots. It is possible that different rates used in the experiments affected the development and spread of the disease or, alternatively, nitrogen rates may have had a different effect on different cultivars and their resistance to the disease. Further work is needed to clarify the effect of nitrogen on the development of the disease caused by *O. acuformis* or *O. yallundae* in modern winter wheat cultivars.

v. Weed control

Cunningham (1966) reported that herbicide application on spring wheat reduced eyespot, compared to the unsprayed control plots. According to the author, herbicide application decreased eyespot severity indirectly by creating less favourable conditions within the crop for the development and spread of the disease.

Isolates of *Oculimacula* spp. have been recovered successfully from several species of grass that are considered weeds, including annual meadow grass (*Poa annua*), couch (*Elytrigia repens*) and cocksfoot (*Dactylis glomerata*) (Lucas *et al.*, 2000). There is little published information on the role of the weeds regarding eyespot development and control and therefore the efficacy of weed control in reducing the disease remains unclear. Further studies to identify common weed species hosting the eyespot pathogens and their

significance as providers of disease inoculum and dispersal are necessary to clarify the potential use of effective weed control in minimising disease incidence or severity.

Genetic resistance to eyespot

Sprague (1936) studied the susceptibility of cereals and grasses to eyespot in greenhouse conditions and identified *Aegilops ventricosa* as one of the potential sources of resistance to the disease. Macer (1966) confirmed this observation in a study of resistance to eyespot determined by inoculations of seedlings of *Triticum*, *Aegilops*, *Secale* and *Hordeum* and noticed that a low degree of resistance existed in cultivars of *Hordeum* whilst cultivars of *Secale* showed higher resistance than *Triticum* or *Hordeum*. Maia (1967) and Kimber (1967) introduced resistance to eyespot from *A. ventricosa* to winter wheat by recombination through a series of crosses. The new winter wheat cultivar which had some of the resistance of *A. ventricosa* was known as VPM1 (Maia, 1967). Before VPM1, the only wheat cultivar with moderate, but stable resistance against eyespot of unknown origin was Capelle Desprez (Vincent *et al.*, 1955; Lupton & Macer, 1955; Lupton & Macer, 1958; Doussinault, 1970). Law *et al.* (1976) showed that the majority of the resistance present in Capelle-Desprez was carried on the chromosome 7AL. The resistance in VPM1 was determined by a single gene located on the chromosome 7DL as shown by F_2 monosomic analysis (Jahier *et al.*, 1979; Worland *et al.*, 1988). The eyespot resistance genes, *Pch*1 and *Pch*2, were identified, the former from *A. ventricosa* (Dossinault *et al.*, 1983) and the latter in Capelle-Desprez (Law *et al.*, 1976; de la Peña *et al.*, 1996). Although *Pch*1 was very effective, conferring higher levels of eyespot resistance than *Pch*2, and was widely used in the breeding of resistant cultivars, it appeared that it indirectly affected agronomic characteristics of winter wheat, typically reducing thousand grain weight, ear yield, and plant yield by 4% (Worland & Law, 1985). This was possibly

a result of the incorporation of a segment of *A. ventricosa* chromosome 7DL that did not readily recombine with *T. aestivum* or, alternatively, Scott *et al.* (1989) suggested that the cause of reduced yield could be cytoplasmic since Dyer & Bowman (1987) had shown that the cytoplasm of VPM1 was that of *A. ventricosa*. Jahier & Lucas (1987) carried out several crosses between *Triticum*, *Aegilops*, *S. cereale* and *Haynaldia villosa* in search of new genes for resistance and found one line in the progeny of the cross *A. ventricosa* x *S. cereale* x *T. aestivum* to have higher resistance to eyespot than VPM1, especially in the seedling stage, indicating that more genes of resistance from rye can potentially be introduced into a new wheat line.

Identification of new eyespot resistance genes, however, has proved to be difficult because of the quantitative nature of resistance (Scott *et al.*, 1989). Indeed, isolates of *Oculimacula* spp. exhibit considerable capacity for pathogenic adaptation to different host species, suggesting that resistance introduced into wheat from other species may be less durable than resistance from within the species (Scott *et al.*, 1976; Scott & Hollins, 1980). The winter wheat cultivar Rendezvous was thought to contain both resistance genes *Pch*1 and *Pch*2, but it still suffered yield loss under severe eyespot epidemics (Hollins *et al.*, 1988), thus emphasising the need for other sources of resistance. More recently, Murray *et al.* (1994) identified a new single, dominant resistance gene, *Pch*3, for eyespot on chromosome 4VL of *Dasypirum villosum*. In addition, Yildrim *et al.* (1998) located another gene for resistance to eyespot from *Triticum tauchii* after screening more than 270 accessions of the species by inoculation of seedlings with genetically modified strain of *O. yallundae* expressing β-glucuronidase (GUS) (Yildirim *et al.*, 1995).

Uslu *et al.* (1998) studied comparative resistance in *D. villosum* to both *Oculimacula* spp. using competitive PCR assays to measure fungal colonisation in a series of *Dasypirum* chromosome addition lines in Chinese Spring wheat. The authors found that lines containing chromosomes 1V, 2V, 4V, and possibly 3V were significantly more resistant to *O. yallundae* than was the susceptible Chinese Spring wheat, whilst lines

containing 1V, 2V, 5V and possibly 3V were significantly more resistant to *O. acuformis* than was Chinese Spring wheat. These differences indicated that, although some of the genes were identical, separate, unique genes might confer resistance to the two eyespot pathogens.

The complexity of resistance to eyespot was recently demonstrated by a study carried out by Muranty *et al.* (2002) whilst investigating the inheritance of resistance of wheat to eyespot at the adult stage. The authors showed that, although chromosome 7A in Capelle Desprez conferred resistance to eyespot at the seedling stage, it had no effect at the adult stage. Furthermore, chromosome 5A was shown to carry a major gene for resistance at the adult stage of winter wheat, which appeared to be stable during the four years of testing.

i. Screening techniques

Techniques to evaluate resistance in wheat genotypes have been based largely on visual assessment of disease severity or lesion index (Macer, 1966; McMillin *et al.*, 1986). Seedling resistance has been measured also by rate of leaf sheath penetration by the fungus (Scott & Hollins, 1977). Strausbaugh & Murray (1989a & b) used epidermal cell responses (papilla formation, penetrations stopped within epidermal cells and hypersensitivity) on the first leaf sheath under different environmental conditions to evaluate differences in resistance of winter wheat seedlings, inoculated with *O. yallundae*. The authors found that the greatest differentiation between highly resistant, moderately resistant and susceptible cultivars was obtained at 10°C, using ratings based on fungal penetrations stopped by papillae or within epidermal cells in both hypersensitive and non-hypersensitive cells. Lind (1992) and Lind *et al.* (1994) used ELISA to differentiate between resistant and susceptible genotypes but differences were best demonstrated at growth stages 65 to 75 with medium levels of disease severity. Alternatively, de la Peña &

Murray (1994) identified resistant wheat genotypes by inoculating 2 week old seedlings with a GUS-transformed strain of *O. yallundae* and measuring disease development with a GUS enzyme assay. Greatest differences among resistant and susceptible genotypes occurred between 4 and 8 weeks after inoculation.

Resistance to eyespot has been associated with morphological and biochemical markers used in mapping and assessing diversity of wild populations (Law *et al.*, 1976). *Pch*1 has been linked with the Ep-V1 endopeptidase allele derived from *Aegilops ventricosa* (McMillin *et al.*, 1986). Gale *et al.* (1984) studied the α-amylase allele, using α-Amy-D2b as a marker for resistance, transferred from *A. ventricosa.* The absence of the α-Amy-D2b in F_3 families from a cross between VPM 1 and a line lacking *Pch*1 and the α-Amylase allele, indicated that the two characters segregated independently, dismissing the α-Amy-D2b as a marker for *Pch*1. Red coleoptile colour has also been associated with *Pch*1 (Worland *et al.*, 1988) and *Pch*2, although, it has been shown that *Pch*2 is not linked with this trait (Law *et al.*, 1976; de la Peña *et al.*, 1996). De la Peña *et al* (1997) identified two flanking RFLP markers Xcdo347 and Xwg380, which were used for the selection of *Pch*2.

Biological control

There are few reports on the biological control of eyespot. However, antagonistic effects between the fungi of the stem base disease complex have been used for manipulations of disease levels. Reinecke *et al.* (1979), for example, used artificial inoculation with *R. cerealis* on winter varieties of wheat and rye to reduce the disease index of eyespot and brown foot rot, caused by *Fusarium* spp., and achieved 62% and 72% reduction, respectively. However, this positive effect was reversed when plots were later treated with

fungicides active against sharp eyespot (benodanil, 2-iodinil-benzoic-acid anilide). Later, Reinecke & Fokkema (1981) used four different methods to screen fungi from the base of cereals for antagonism to *Oculimacula* sp. Using winter wheat seed inoculated with *Oculimacula* sp. and *Microdochium bolleyi* as potential antagonist and grown in sterile sand, the researchers observed up to 80% reduction in disease index, where *M. bolleyi* was applied at 10 times higher concentration than the pathogen. Other identified fungal antagonists to both *Oculimacula* spp. were several *Trichoderma* spp. (Clarkson & Lucas, 1993; Hinton & Parry, 1993). Clarkson & Lucas (1993) screened *in vitro* and *in vivo* several potential bacterial antagonists of both eyespot pathogens and identified two isolates of *Pseudomonas fluorescens* and one commercial strain of *Streptomyces griseoviridis* as effective suppressors of eyespot disease. In a later study, Clarkson & Lucas (1997) identified two antibiotic compounds, produced by a single isolate of *Ps. fluorescens* in nutrient broth, which were inhibitory to spore germination and hyphal growth of *O. acuformis*. These effects, however, were studied using just one isolate of *O. acuformis* and no isolates of *O. yallundae,* thus providing limited information.

Chemical control

Chemical control of eyespot has been shown to be inconsistent and there are three reasons to explain this variability. First, the active ingredients of many fungicides used for eyespot control have been shown to have a differential effect on the two eyespot fungi, because of differences in resistance or intrinsic sensitivity. There are also problems associated with the timing of fungicide application, especially when eyespot is caused by *O. acuformis*, which tends to develop later in the season than *O. yallundae*. Last, eyespot is part of a stem base disease complex, consisting of two other diseases, brown foot rot and sharp

eyespot. Symptoms of these three diseases can be similar at early crop growth stages, when decisions to spray for eyespot control are made. In addition, often when fungicides have good activity against one of the stem base diseases, this effect is masked by the subsequent development of the others (See **The stem-base disease complex, identification, diagnosis and interactions between diseases and their pathogens**). The most common treatments used for the control of eyespot are listed in Table 1.1.

i. Fungicide resistance in the eyespot population

The widespread use of the MBC fungicides (e.g. benomyl, carbendazim) during the period 1970–1980 led to acquired, qualitative resistance in both *Oculimacula* spp. (Leroux & Gredt, 1997; Leroux *et al.*, 2003). MBC-resistant isolates of *Oculimacula* spp. were found in Germany (Fehrmann *et al.*, 1982), UK (Brown *et al.*, 1984; King & Griffin, 1985), Belgium (Maraite *et al.*, 1985), The Netherlands (Sanders *et al.*, 1986) and France (Leroux & Gredt, 1997). The resistance in both species was due to similar base pair mutations in the gene encoding for β-tubulin (Albertini *et al.*, 1999). The development of persistent resistance led to the withdrawal of benzimidazoles for use against eyespot.

The second group of fungicides used for eyespot control were the sterol 14α - demethylase inhibitors (DMIs), such as the imidazole, prochloraz, and many triazoles including bromuconazole, cyproconazole, epoxyconazole and flusilazole. *In vitro* tests, using wild type isolates of eyespot fungi have shown that the population of *O. acuformis* was intrinsically less sensitive towards most DMIs in comparison to *O. yallundae* (Leroux & Gredt, 1997). Following five years of field trials on the comparative efficacy of flusilazole or other triazoles and prochloraz on eyespot epidemics caused predominantly by *O. acuformis*, Leroux & Gredt (1997) determined that the selection pressure exerted by triazole derivatives towards *O. acuformis* was greater than by prochloraz. However, the

extensive use of prochloraz, which was initially effective against both species, led to the development of practical resistance in the population of *O. acuformis* in France (Leroux & Marchegay, 1991) and decreased sensitivity to prochloraz in populations, studied in the UK (Bateman, 2002). Recently, isolates of *O. yallundae* have been detected in France with acquired quantitative resistance to triazoles and isolates which are resistant to both triazoles and prochloraz (Leroux & Gredt, 1997). Robbertse *et al.* (1996) also reported the detection of isolates of *O. yallundae* in South Africa resistant to triadimenol and tebuconazole.

More recently, Dyer *et al.* (2000) used sexual crosses between sensitive and resistant isolates of *O. yallundae* to determine the genetic nature of resistance to prochloraz. The progeny of these crosses showed bimodal distribution with an even number of sensitive and resistant progeny, indicating the segregation of a single major gene. Further work by Wood *et al.* (2001) showed that the resistance to prochloraz was not related to sequence mutations in the gene *CYP51* encoding the target enzyme eburicol 14α – demethylase, as previously thought. Thus, more research is needed to establish the exact genetic basis of prochloraz resistance. Albertini *et al.* (2003) also failed to find a clear relationship between any mutations in *CYP51* and resistance in *Oculimacula* spp. The authors, however, found that a conserved phenylalanine residue at position 180 found in *O. yallundae* and in all known *CYP51* proteins from filamentous fungi and yeast was replaced by leucine in *O. acuformis* and suggested that this change could be implicated in the natural resistance of *O. acuformis* to triazoles.

A third fungicide, belonging to the anilinopyrimidine group used for the control of eyespot is cyprodinil. Cyprodinil has shown good efficacy for the control of eyespot (Bateman *et al.*, 2000a; Burnett *et al.*, 2000). However, Babij *et al.* (2000) detected isolates of both *Oculimacula* spp. with reduced sensitivity to cyprodinil in field trials, sprayed for more than 11 consecutive years with cyprodinil. Analysis of *O. yallundae* progeny from sexual crosses between sensitive and field isolates with reduced sensitivity

indicated that a single gene controlled the reduction of sensitivity to cyprodinil. In addition, cyprodinil resistant field isolates of both species have been recently detected in France (Leroux *et al.*, 2003).

ii. Timing of fungicide application

Fungicide applications for the control of eyespot are conventionally applied between GS 30 and 32 (Anon., 1986). From the published literature it is clear that different fungicides have different optimum timings for the effective control of the disease. For example, MBC fungicides were more effective in reducing the disease severity early in the season at GS 30/31, while other fungicide treatments such as prochloraz allowed greater flexibility in spray timing up to GS 37 (Goulds & Fitt, 1990b). The efficacy of prochloraz in controlling the disease at later growth stages was associated with adequate rainfall following fungicide application aiding the redistribution of prochloraz from the foliage to the plant base (Cooke *et al.*, 1989; Cooke *et al.*, 1993). Daniels & Lucas (1990) used microscopic techniques to assess the effects of prochloraz on early pathogenesis of the eyespot fungi and reported that prochloraz applied as protectant spray was more effective in controlling the disease than as eradicant spray, because of re-growth of the pathogen from structures within host tissues not directly exposed to the fungicide. Jones (1995) carried out a series of field experiments investigating the optimum timing of prochloraz and determined, that the most consistent control of eyespot by prochloraz was obtained by application at GS30-33 rather than GS 37-39. Differences in the control of eyespot due to causal species as well as time and type of fungicide application are important if eyespot disease is to be effectively controlled. Burnett *et al.* (1997) used PCR assays to study the effects of prochloraz and cyprodinil on both *Oculimacula* spp. These researchers found that using prochloraz early in the season, during tillering (GS25), reduced significantly the *O. acuformis* population but allowed the *O. yallundae* population to recover, exceeding the DNA amounts in untreated controls by

6%. Conversely, cyprodinil achieved greatest reduction in fungal populations when applied later, after the start of stem extension (GS31), because earlier application led to the recovery of fungal populations, in particular that of *O. yallundae*. Later, Burnett (1999) reported that using split treatments of prochloraz or cyprodinil failed to improve eyespot control compared to single applications of the fungicides at their optimum timings.

Several forecasting systems have been developed to predict the occurrence of severe eyespot at a time when spray decisions need to be made (Fehrmann & Schrödter, 1973; Van der Spec *et al.*, 1974; Polley & Clarkson, 1978; Jørgensen *et al.*, 1996). It has proved difficult, however, to predict the magnitude of losses due to eyespot at the time that spray decisions are made because of many limiting factors, including unpredictability of summer weather and the occurrence of additional losses due to lodging.

Table 1.1. Summary of the most common fungicides used to control eyespot in winter wheat experiments or *in vitro* fungicide tests, with examples of research and efficacy.

Active ingredient	Details of experiment	Notes	Reference
Benomyl, thiabendazole	Naturally infected, field trials	Treatments applied as sprays or seed treatments decreased eyespot	Prew & McIntosh (1975)
Prochloraz	*In vitro* tests on amended media using 224 isolates	Isolates of *O. yallundae* were significantly less sensitive than isolates of *O. acuformis*	Birchmore *et al.* (1986)
Prochloraz	*In vitro* tests on amended media using 86 isolates of *O. yallundae* and 105 of *O. acuformis*	Isolates of *O. acuformis* had narrower sensitivity range than isolates of *O. yallundae*	Gallimore *et al.* (1987)
Prochloraz and cyproconazole	Three years of naturally infected field trials	Co–formulation mixture applied at GS 31increased yield on winter wheat by average of 8.9 % and decreased eyespot by average of 54.5% compared to untreated control	Bush & Bardsley (1988)
Prochloraz, flusilazole	Forty naturally infected field experiments on winter wheat	Prochloraz reduced eyespot index by 30-60%, flusilazole was as effective as prochloraz	Jones (1994)
Tebuconazole, prochloraz, carbendazim	Three naturally infected field experiments on winter wheat	Carbendazim inclusive mixtures and prochloraz reduced lodging due to eyespot significantly compared to untreated control	Scott (1996)
Prochloraz, flusilazole, fenetrazole, cyproconazole	Glasshouse experiments with inoculated winter wheat plants with both *Oculimacula* spp.	Prochloraz was effective against both species, all other treatments were more effective against *O. yallundae* than *O. acuformis*	Cavalier (1998)
Cyprodinil	Naturally infected field experiments on winter wheat	Treatment decreased eyespot, which was reflected in an increased numbers of fertile tillers per m^2 and number of grains per ear, by 8 and 14%, respectively	Stadnik & Buchenauer (1999)
Cyprodinil, prochloraz, azoxystrobin, flusilazole	Naturally infected field experiments on winter wheat	Cyprodinil was most effective in reducing pathogen DNA and symptoms of eyespot, caused by both species, prochloraz was less consistent and more effective against *O. yallundae*	Bateman *et al.* (2000)
Prothioconazole	Naturally infected field experiments on winter wheat	Provided 65% control of eyespot	Mauler-Machnik *et al.* (2002)

Effects of eyespot on yield

Yield losses from eyespot are associated with direct effects of lesions, which interfere with the movement of water and nutrients through the stem and with the indirect effect of lodging (Glynne, 1944; Scott & Hollins, 1974; Fitt *et al.*, 1988). Experiments using artificial inoculation provided a great deal of information on the extent of yield losses attributable to the direct effects of eyespot. In the absence of lodging, Oort (1936) and Glynne & Salt (1958) reported that winter wheat grain losses were 25% and 33%, respectively. A wealth of information is also available regarding the type of eyespot lesions causing yield loss. Losses in grain yield per ear are attributable mainly to severe or moderate lesions and not to slight lesions (Jørgensen, 1964b; Scott & Hollins, 1974; Clarkson, 1981). Jørgensen (1964b) reported reductions in grain weight per ear of 15 and 40% caused by moderate and severe eyespot lesions, respectively. Similarly, Clarkson (1981) showed that moderate and severe eyespot lesions reduced yield per ear by 10% and 36%, respectively.

With or without lodging, eyespot also reduced grain number per ear and thousand grain weight. Scott & Hollins, (1974) and Clarkson (1981) demonstrated that severe lesions could cause reductions of up to 29% in grain number per ear. Moderate lesions have been shown to reduce thousand grain weight by 3 to 5%, whilst severe eyespot lesions caused corresponding losses of 12 to 15% (Defosse & Rixhon, 1968; Scott & Hollins, 1974; Clarkson, 1981). In the presence of lodging, yield loss caused by eyespot on winter wheat was always greater. Furthermore, Scott & Hollins (1978) demonstrated that yield loss in their inoculated field experiments was related more closely to the amount of lodging due to eyespot than to the incidence of severe eyespot. Eyespot-induced lodging has been shown to be more damaging when it occurred earlier in the crop's

growing season (Glynne, 1944). However, lodging generally also depends on other factors such as weather (wind and rain), soil fertility and nitrogen inputs, and the resistance of the cultivar to lodging (Scott & Hollins, 1978). Thus it is difficult to predict the occurrence or the relative contribution to yield loss made by direct effects of the disease and eyespot lodging. Nonetheless, inoculated experimental crops provided direct measure of yield loss when lodging occurred. In a pot experiment where all inoculated wheat tillers were diseased and 86% lodged, Glynne *et al.* (1945) recorded yield loss of 44%. Jørgensen (1964) reported an average of 40% yield reduction in inoculated susceptible cultivars in which 95% of tillers became infected and lodged. Scott & Hollins (1974) carried out inoculated experiments where wheat plants were supported or left to lodge and demonstrated by regression analysis that yield loss was significantly greater in the unsupported plots due to eyespot-induced lodging than in the supported plots where plants were prevented from lodging.

Relationships between yield loss, incidence and severity of eyespot and pathogen DNA

There are many difficulties in measuring the loss in yield due to eyespot. First, it is often difficult to induce eyespot attacks of different severity in the field, because of the dependence of disease on favourable environmental conditions. Fitt & White (1988) considered the progress of eyespot epidemics in four stages: leaf sheath lesion establishment, leaf sheath penetration, stem lesion establishment and stem lesion development. There is considerable evidence that the penetration of leaf sheaths by the eyespot fungi is dependent on temperature (Pochet, 1959; Rapilly *et al.*, 1979). For example, the rate of leaf sheaf penetration has been shown to increase with temperature

over the range 6-18°C (Scott, 1971; Fitt, 1985). However, the first two stages are also influenced by the production of new inner leaf sheaths by the plant and the death and disintegration of outer leaf sheaths with the latter process being enhanced by either cold (average temperature 2.5 °C) (Fitt, 1985) or warm dry weather (average temperature 13°C, 0.2 mm rainfall) (Higgins *et al.*, 1986). Thus, lesions may fail to develop if the rate of leaf sheath penetration is slower than the rate of leaf sheath production or if death rate of leaf sheaths is greater than the penetration rate of the fungus (Fitt *et al.*, 1988). Higgins *et al.* (1986) established that the time after stem extension, when the fungus is spreading from the innermost leaf sheaths to the stem, is a crucial stage in the development of eyespot. This period of stem lesion establishment is also influenced by environmental factors. Higgins *et al.* (1986) noticed that warm, dry weather following stem extension impaired stem lesion establishment in an early drilled winter wheat, where 75% of plants had infected leaf sheaths when stem extension began. Thus, severe stem lesions develop only after a period of successful lesion establishment (Fitt, 1985).

When eyespot lesions first become visible on leaf sheaths of plants in winter wheat crops, the incidence of eyespot is assessed as the proportion of plants or shoots with visible lesions (Fitt *et al.*, 1988). Eyespot severity is assessed by the depth of penetration of the lesions at the base of the shoots and it is often expressed on a 0-3 scale (Scott & Hollins, 1974). Relationships between incidence and severity of eyespot are often more complicated due to the assessment methods, which are often considered imprecise because the optimal size of the samples and best method of sampling are unclear (Fitt *et al.*, 1988). Scott & Hollins (1974) used severity index to incorporate both incidence and severity of the disease in order to minimise complications arising from changes in both measures of the disease between successive samples.

Attempts have been made to quantify the relationship between eyespot severity and yield loss. Glynne (1963) used extrapolation from regression on disease incidence in winter wheat experiments to estimate that severe infection of all shoots would reduce yield

by 50%. Using multiple linear regression, Scott & Hollins (1978) derived an equation, which accounted for percentage yield loss (y) in winter wheat caused by the incidence of severe lesions (x_1) and lodging index (x_2), $y = -0.19 + 0.17x_1 + 0.41x_2$. Lodging index alone, however, accounted for much of the variation for yield loss. Following nine inoculated winter wheat experiments, these researchers showed that assessments of incidence or severity in spring (GS32) were not reliable indicators of subsequent disease development, but observed that in experiments where in spring there were fewer than 10% diseased shoots, the costs of treating the disease with fungicide were not recovered. Much of the work on relationships between yield loss and eyespot severity was carried out on single shoot basis, which may not be representative of yield loss per plant, due to compensation effects by healthy or less infected tillers (Scott & Higgins, 1974). Clarkson (1981) analysed large numbers of individual stems taken before harvest from commercial winter wheat crops. From his results, he derived an equation, accounting for percentage yield loss (y) in winter wheat caused by severe and moderate eyespot lesions: $y = 0.1x_1 + 0.36x_2$, where x_1 is the percentage of moderately infected shoots and x_2 is the percentage of severely infected shoots. Based on this equation, annual yield losses due to eyespot for the period between 1985 and 1989 in England and Wales were estimated at 1.5% (Cook *et al.*, 1991) and for the period of 1989 to 1998 at 1.2% (Hardwick *et al.*, 2001). However, these figures do not account for yield losses due to eyespot-induced lodging and, although useful in assessing losses in retrospect they can not be used for the prediction of losses at the time when spray decisions are made (Fitt *et al.*, 1988).

The threshold level for eyespot was set at 20% of tillers with penetrating severe lesions at GS 30-32 (Anon., 1986). This threshold was based on work carried out in the 1970s and 1980s (as discussed above) on the relationships between yield loss and severity of eyespot for winter wheat. In much of the experimental work done to determine these relationships, the species causing the disease is uncertain. However, since at the time *O. yallundae* was the dominant pathogen in the eyespot population in the UK, it is generally

accepted that these relationships were valid for eyespot epidemics caused by this pathogen and not necessarily caused by *O. acuformis*. Presently, it is unclear if yield loss and disease severity relationships as determined in the 1970s and 1980s are still applicable to disease epidemics caused by *O. acuformis*.

Goulds & Fitt (1991a) compared several methods for measuring eyespot incidence/severity at different growth stages to predict eyespot severity at grain filling of winter wheat and barley inoculated or *O. yallundae* and *O. acuformis*. They found that prediction of eyespot severity during grain filling of winter wheat or barley was more difficult for disease caused by *O. acuformis* than by *O. yallundae* at early growth stages. In both crops, improvement in prediction of severity of disease caused by *O. acuformis* was made when assessments were made at later growth stages of 37/39. Goulds & Fitt (1991b) determined that differences between species were based on their rates of development on seedlings and on adult plants. The development of *O. acuformis* on seedlings was not a reliable predictor of disease development on adult plants. Furthermore it appeared that the progress of epidemics caused by *O. acuformis* was influenced more by favourable weather than epidemics by *O. yallundae*. Jones (1994) evaluated the applicability of the eyespot threshold on 40 sites of winter wheat and found that, although fungicide applications were cost effective for 60% of the sites, there was a weak relationship between yield loss and eyespot incidence or severity at either GS31 or GS 75, indicating that eyespot assessments in spring were not reliable indicators of disease progression or subsequent yield loss. From this work, however, it is not clear how the different species affected the applicability of the threshold. Later, Royle (1998) used ELISA and PCR to monitor the progress of eyespot epidemics in winter wheat caused by *O. acuformis* or *O. yallundae*. Both techniques detected eyespot pathogens pre–symptomatically. However, correlations between antigen or DNA concentrations of both pathogens and final disease or yield of winter wheat were weak. Other researchers have also found little correlation between DNA concentration of *O. acuformis* early in the

season and eyespot severity or final yield of winter wheat (Turner *et al.*, 1999; Bateman *et al.*, 2000a & b; Turner *et al.*, 2001). These researchers observed that *O. acuformis* developed later in season than *O. yallundae,* thus symptoms were not easily identified at early growth stages, especially where other stem-base diseases were present.

AIMS OF THE PROJECT

1. To determine the potential of each *Oculimacula* spp. to cause yield loss or lodging in winter wheat and re-examine disease severity and yield relationships for eyespot caused by *O. acuformis*.
2. To determine the effect of fungicide applications on eyespot caused by *O. acuformis* and yield of early-drilled winter wheat crops.
3. To determine the role of *Microdochium nivale*, frequently occurring simultaneously with *Oculimacula* spp. on wheat stem bases, on the development of eyespot and *O. acuformis* and *O. yallundae* throughout the season.

The objectives of the research described in this thesis were to:

i) To compare the progress of eyespot disease when caused by *O. acuformis* or *O. yallundae* and to determine the effects of disease on yield of individual plants and tillers of winter wheat.

ii) To identify any relationships between pathogen DNA concentration, eyespot severity and lodging risk in winter wheat.

iii) To assess the effects of current fungicides on pathogen DNA concentration, the severity of eyespot and yield of winter wheat.

iv) To identify any relationships among the pathogens of stem base diseases and their effect on eyespot disease caused by *O. acuformis* or *O. yallundae*.

CHAPTER 2

GENERAL MATERIALS AND METHODS

GENERAL MATERIALS AND METHODS

Fungal isolation and maintenance

All aseptic operations were carried out in a sterile flow cabinet. Glassware, media and sterile distilled water (SDW) were autoclaved at 121 °C and 103.4 KPa for 20 minutes. Plant bases cut to 1 cm small pieces were surface sterilised in a sodium hypochlorite solution (1.2 % available chlorine) for 3 min. The pieces were rinsed in three changes of SDW and placed in an open Petri dish (Sarstedt, UK) in a laminar air flow to dry and incubated on potato dextrose agar (PDA, 39 g l^{-1} of distilled water) (Merck, Germany) amended with streptomycin sulphate (69.5mg l^{-1} of agar) or chloramphenicol (5mg l^{-1} of agar), or rose-bengal chloramphenicol agar (RBA, 32.2 g l^{-1} of distilled water) (Merck, Germany) for up to 3 weeks. Emanating colonies of *Oculimacula* spp. were transferred to fresh PDA or malt yeast glucose agar (MYG) as small pieces of mycelium. Table 2.1 lists the fungal isolates used during the experimental work described in this project. All isolates were obtained from Harper Adams University College culture collection.

Table 2.1. Isolates used in the experimental work.

Species	Isolate	Origin
Oculimacula acuformis	130/4, 159/7,162/3	Berkshire, Yorkshire, Shropshire
Oculimacula yallundae	109/13,136/8,166/3	Northumberland, Kent, Shropshire
Rhizoctonia cerealis	97/6	Northantshire
Microdochium nivale	30/1,74/1,117/1	Harper Adams University College

Mycelial plugs from active growing cultures were placed onto tap water agar (TWA) and incubated at 10°C under near-ultra violet (NUV) light for 3-6 weeks to induce sporulation. A small quantity of spores was removed from each culture using a sterile microbiological

loop and mixed in 1 ml SDW, spread onto a PDA plate and incubated at 20°C. After 48 h, germinating, single conidia were identified under a binocular field microscope and transferred to fresh PDA plates and incubated at 20°C prior to species identification.

For medium–term maintenance a 5 mm mycelial plug taken from the edge of an actively growing culture was transferred to a sterile universal bottle containing approximately 10 ml of 2% PDA, which was allowed to set at an angle of approximately 45 °. The cultures were incubated at 20°C for 7 days to allow the culture to develop before being stored at 4 °C. Single-spore cultures of identified isolates were maintained long-term as spore suspensions in 10% glycerol at -80 °C.

Several types of artificial media were tested for optimum growth of cultures (Appendix 1). Consequently MYG agar, maize agar and PDA amended with streptomycin sulphate were used for sub-culture and short-term maintenance at 20 °C.

Inoculum preparation and application

The method for production of oat-grain inoculum was modified from Bruehl & Nelson (1964).

Oat-grain inoculum

Isolates of *O. yallundae* and *O. acuformis* were grown on PDA at 20°C for 5 weeks. Oat grains (300 g) and 240 ml of distilled water were placed in an autoclave bag and were autoclaved for 1 h on two consecutive days. Five mycelial plugs (5 mm) from each isolate were added to a bag of oats in a sterile laminar flow cabinet. Incubation took place at 20°C for approximately 6 weeks.

Plants in field were always inoculated at GS 12 (two leaf stage) (Zadoks *et al.*, 1974). Oat inoculum was spread manually over the plots at 6 g m^{-2}.

Fungicide application in field experiments

Fungicides were applied in the field using a pressurised knapsack sprayer (AZO carbon dioxide operated sprayer, AZO sprayers, The Netherlands) with a six-nozzle boom. Fungicides were applied at 3 bars pressure (1.2 l min^{-1}), in 180 l of water ha^{-1} using Lurmark 110 ° flat fan nozzles (F110/02), medium/fine spray quality. Fungicides tested in field experiments are listed in Table 2.2.

Table 2.2. Fungicides used in field studies.

Active Ingredient		**Product Name**	**Maximum Field Rate**	**Manufacturer**
Epoxiconazole	125 g l^{-1}	Opus SC	1 l ha^{-1}	BASF
Kresixim methyl + Epoxiconazole	125 g l^{-1} + 125 g l^{-1}	Landmark SC	1 l ha^{-1}	BASF
Prochloraz	450 g l^{-1}	Sportak 45 EW	0.9 l ha^{-1}	Bayer Crop Science
Fluquinconazole + Prochloraz	54 g l^{-1} + 174 g l^{-1}	Foil SE	0.9 l ha^{-1}	Bayer Crop Science
Cyprodinil	75% w/w	Unix WG	1 kg ha^{-1}	Syngenta

Disease assessments

For all experiments, disease assessments were made immediately after sampling. Disease assessments for incidence and disease index (DI), representing disease intensity, were made for eyespot, sharp eyespot, and brown foot rot at different plant growth stages. The method of disease assessment was adapted from Scott & Hollins (1974) and Goulds & Polley (1990) and disease on assessed plants was recorded as slight, moderate or severe (Appendix 2). The main shoot and all tillers were used for assessment at the early growth stages (30 to 55, see Appendix 2), after growth stage 55 assessments were carried out on the main shoot only. It was expected that by including all plant tillers in the assessment of the disease at early growth stages we might improve the diagnosis of incidence and severity of eyespot on a whole plant basis. Incidence was recorded as percentage of the assessed plants showing symptoms of the disease, while DI was calculated using the following formula:

DI = ((number of plants with slight symptoms) + (2 x number of plants with moderate symptoms) + (3 x number of plants with severe symptoms)) / (3 x Total number of plants assessed) x 100

Where applicable, the same method of disease assessment and categories were used for sharp eyespot and brown foot rot.

DNA extraction and measurement from fungi

Method 1

A small amount of mycelium was removed from a fungal colony using a sterile microbiological loop and placed into a sterile 1.5 ml Eppendorf tube. Chelex carbon buffer (0.25 ml) (Appendix 3) was added and the mycelium was crushed using a sterile

micropestle. The tubes were vortexed for 10 s and kept at 56 °C for 10 min and left to cool before being centrifuged at 12000 x*g* for 15 min. The supernatant (100 µl) was removed and placed into a fresh tube and diluted two-fold in TE buffer (Appendix 3) for use in PCR reactions.

Method 2

A small amount of mycelium was removed from a fungal colony using a sterile microbiological loop and placed into a sterile 1.5 ml Eppendorf tube. CTAB buffer (600µl) (Appendix 3) was added and the mycelium was crushed with micropestle. The tubes were placed quickly 3-4 times in a bath containing dry ice and ethanol and then into a heating block (Grant QBTP, Grant Instruments Ltd, UK) at 65 °C. Following incubation at 65 °C for 1 hour, chloroform (600µl) was added to the tubes, which were then vortexed and centrifuged at 12000 x*g* for 15 min. The supernatant (400µl) was removed and placed into a fresh tube, isopropanol (600µl) was added to the tubes, which were mixed by inversion and then left at room temperature for 10 min to allow DNA precipitation. Tubes were centrifuged at 12000 x*g*, the resulting DNA pellets were washed twice in isopropanol (44%) and left to air-dry. DNA was dissolved in TE buffer (50µl). A 20 µl sub-sample was diluted ten-fold for DNA quantification using a DUK 400 spectrophotometer (Beckman Instruments Inc., Fullerton, USA). Absorbance was measured at 260, 280, and 320 nm. The final concentration of DNA in each sample was determined using the Warburg Christian coefficient (Anon, 1992). Samples were diluted to a concentration of 1 ng μl^{-1} for use in PCR reactions.

DNA extraction from plants

The basal region (4 cm length) of each plant stem was chopped finely, placed in 50 ml kartell tubes (Fisher, UK) and frozen for 16 h before being freeze-dried in a Modulyo freeze drier (Edwards, UK). After 5 days, the tubes were removed from the freeze drier and four sterile stainless steel ball bearings (one with 22 mm diameter and three with 8 mm diameter) were placed in each tube and shaken using a soil mill (Griffin, UK) for 1-2 h. DNA was extracted from the milled plant sample in 30 ml CTAB buffer (Appendix 3) at 65 °C for 1-2 h. Ten ml of 5M potassium acetate (BDH Laboratory Supplies, UK) was mixed in and the samples were frozen for 1 h at -20 °C. The samples were thawed, mixed and centrifuged (3,000 x*g*, 15 min). A 1.3 ml sub-sample of supernatant was removed and added to 0.6 ml chloroform in a 2 ml Eppendorf tube. The contents were mixed by gentle inversion for 1 min and then centrifuged (12,000 x*g*, 15 min). A 1 ml sub-sample of the aqueous phase was transferred to a fresh tube containing 0.8 ml 100% isopropanol. The tubes were gently inverted for 1 min, incubated at 18 °C for 30 min and then centrifuged (6,000 x*g*, 15 min). The resulting DNA pellets were washed twice with 44% isopropanol and then air-dried. The pellets were dissolved in TE buffer (Appendix 3) (200 µl) at 65 °C for 1 h before being stored at 4°C. Samples were diluted to a concentration of 40 ng μl^{-1} for use in PCR reactions.

Diagnostic and quantitative PCR

Primers and PCR conditions

Primer sequences for the specific amplification of *Oculimacula* spp. (Beck *et al.*, 1996), *R. cerealis* (US patent No. 6,485,907), *M. nivale* (JB612 forward primer & ITS-4 reverse primer) and *Fusarium* spp. (JB566 forward primer & JB572 reverse primer) (US Patent

No. 5,815,453, Beck, 1997) were obtained from Jim Beck, Syngenta Biotechnology Inc. Primer details are listed in Appendix 5. Diagnostic PCR reactions were completed in 25 µl volumes and contained 5 µl of the diluted DNA sample. Quantitative PCR reactions were carried out in 50 µl volumes and contained 10 µl of the diluted sample and 10 µl of the appropriate internal standard. A PCR reaction buffer (Appendix 4) was added together with 100 nM of each forward and reverse primer (Appendix 5), and one unit of Taq DNA polymerase (New England BioLabs (UK) Ltd, UK) per 50 µl reaction. Different annealing temperatures were tested for the JBR, JBW and JBSE primers in order to optimise the efficiency and stringency of the amplification. PCR programmes and conditions used for the detection and quantification of specific species can be seen in Appendix 6.

Amplification was performed in a PTC-100 thermal cycler (MJ Research Inc., USA), the PCR programme used for amplification varied depending on the primers used (Appendix 6). PCR products (10 µl) were electrophoresed through agarose gels (2.0 % w/v), prepared using TAE buffer (Appendix 3) and containing 0.05 mg of ethidium bromide per 100 ml TAE buffer.

Electrophoresis and analysis of PCR products

PCR products were visualised following gel electrophoresis using a Gel Doc 1000 fluorescent gel documentation system (Bio-Rad Laboratories Ltd, UK). For quantitative PCR, the intensity of PCR product was determined by analysis of unsaturated images using Molecular Analyst 1.5 software (Bio-Rad). The ratio of intensity of fungal PCR product to an internal standard PCR product was determined for each sample and the amount of fungal DNA determined by interpolation from the relevant standard curve. Samples with saturated images, having too much target DNA, were diluted and quantification was repeated.

Internal standards, JBW, JBR, and JBSE were produced for all primer sets. Other internal standards, JBM and JBF, (developed by S. G. Edwards) were used for quantification of *Microdochium nivale* and *Fusarium* spp., respectively. In addition, a set of primers and internal standard for *Gaeumannomyces graminis* var. *tritici* (developed by R. Wilson, Harper Adams University College, UK) were used in one experiment where severe take-all disease was observed.

The internal standards JBW, JBR and JBSE were constructed from a 1.2 kb fragment of the alliinase gene (EMBL accession code L48614) from onion (*Allium cepa*) based on the method of Förster (1994). Linker primers were designed to obtain internal standards of different sizes to the fungal PCR products and to each other. The fragment was amplified using primers ONI/F and ONI/R (Appendix 5) using the same reaction conditions described above and PCR Programme A (Appendix 6). The 1.2-kb PCR product was excised from the gel after electrophoresis, placed in 1 ml of TE buffer and incubated at 4 °C for 16 h. The gel-slice solution (5 µl) was amplified with linker primers (*O. yallundae*: WL/F, WL/R; *O. acuformis*: RL/F, RL/R; *R. cerealis*: SEL/F, SEL/R; Appendix 5) with a 'Touchup' PCR programme (Appendix 6), consisting of 10 cycles with annealing temperatures of 38°C, followed by 20 cycles with an anneal temperature of 50°C. The resulting PCR products (506 bp, 563 bp and 615 bp for *O. yallundae*, *O. acuformis* and *R. cerealis*, respectively) were excised from the gel after electrophoresis and each was placed in 1 ml TE buffer and incubated for 16 h at 4 °C. These linker products consisted of 486 bp, 543 bp and 595 bp of aliinase gene bordered by the first 10 bp of *O. yallundae*, *O. acuformis* and *R. cerealis* primers. The gel-slice solution (5 µl) was amplified with JBW, JBR and JBSE primers using the 'Touchup' programme. The resulting PCR products consisting of 486 bp, 543 bp and 595 bp of aliinase gene, bordered by the complete primer sites for *O. yallundae*, *O. acuformis* and *R. cerealis*, respectively,

were electrophoresed in a low-melting temperature gel (Kramel Biotech, UK), exised from the gel and purified using Wizard® PCR Prep Kit (Promega, UK) according to the manufacturer's instructions. Purified PCR products were quantified by comparison with Lambda DNA (Sigma) standards and ligated into a pGEM® - T Vector (Promega). The plasmid was transformed into *Ecsherichia coli* JM109 according to the manufacturer's recommendations. Successful transformations were confirmed by white colonies and amplification with JBW, JBR and JBSE primers. A selection of positive clones was grown overnight in LB Broth (Merck) before plasmid DNA was extracted using a Wizard® Plus SV Minipreps DNA purification system (Promega), according to the manufacturer's recommendations. The eluted DNA was diluted in TE buffer and the concentration determined using spectrophotometry.

Production of standard curves

Ten-fold dilutions of fungal target DNA were made and amplified with the respective primers. The concentration of DNA two dilutions before that which gave the last visible band was chosen and 16, two-fold dilutions were made from it. Similarly, internal standard DNA (JBW, JBR, and JBSE) was diluted ten-fold, to a concentration two dilutions before the last visible band and ten two-fold dilutions were made. From the internal standard dilutions, the middle dilution was chosen and co-amplified with the series of fungal standards dilutions. The concentration of internal standard DNA, which allowed the amplification of fungal DNA over the widest range and with greatest sensitivity, was selected. Internal standard DNA was prepared in TE buffer in the presence of 10 ng μl^{-1} carrier (Herring sperm, Sigma) DNA to improve DNA stability during storage at -20 °C. Standard curves were produced for each internal standard and respective fungal DNA (Figure 2.1). The size of the internal standards and fungal products and the limits and range of detection and concentration of internal standards are shown in Table 2.2. Fungal

DNA was diluted two fold over the range specified in Table 3. Ten µl of each dilution was added to separate quantitative reactions together with 10 µl of internal standard of specified concentration (Table 2.3) to produce a standard curve. On the same PCR plate, 10 µl of sample DNA were placed in individual wells with the same amount of internal standard. All constituents used for diagnostic PCR were adjusted to a final reaction volume of 50 µl for quantitative PCR.

Table 2.3. Details of quantitative PCR assays for JBR, JBW and JBSE primers and internal standards.

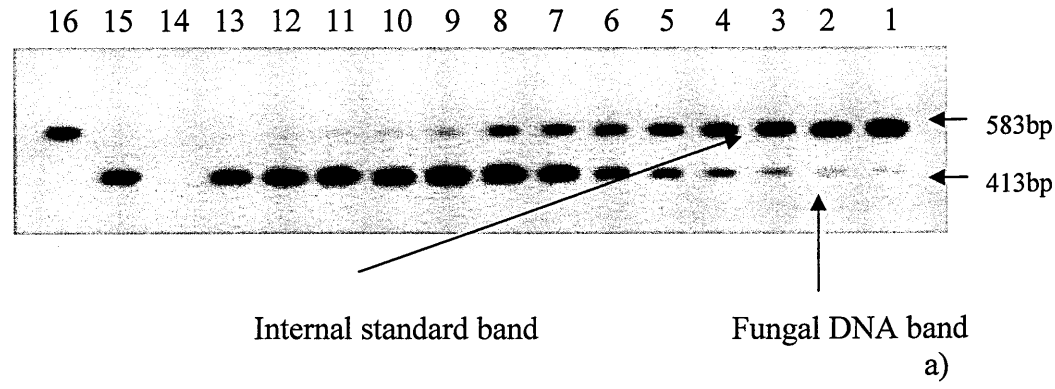
Primers	Species	Size of internal standard (bp)	Size of fungal product (bp)	Detection range (ng μl^{-1})	Concentration of internal standard (fg μl^{-1})
JBR	*O. acuformis*	583	413	1 – 0.00024	258
JBW	*O. yallundae*	526	413	1 – 0.00024	109
JBSE	*R. cerealis*	635	541	1 –0.00097	739

Fungal DNA preparation for quantitative PCR

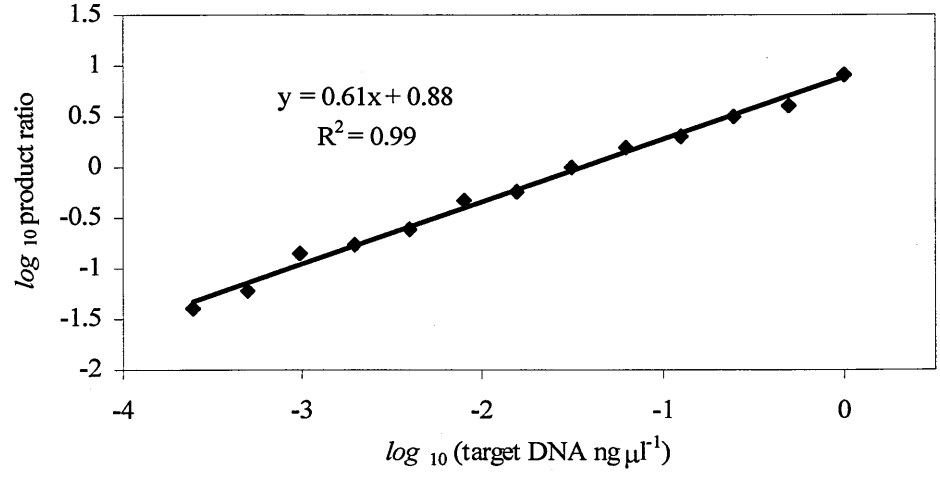
Three-week-old cultures of *O. yallundae, O. acuformis* and *R. cerealis* in malt yeast glucose broth, grown at 19 °C while shaken at 100 rpm, were filtered through sterile muslin, washed with SDW and freeze-dried. DNA was extracted the same as for DNA extraction from plants. Extracted DNA was diluted, following quantification by spectrophotometry and kept at 4°C in batches of 1 ng μl^{-1} stock.

Statistical analysis

All data were analysed using either analysis of variance or regression analysis using Genstat® Version 4.1 for Windows (Lawes Agricultural Trust, UK).



a)



b)

Figure 2.1. Standard curve of fungal DNA and internal standard DNA for JBR primers. (a) PCR products: lanes 1-13, two-fold dilutions of fungal target DNA from 0.00024 ng μl^{-1}-1 ng μl^{-1}, lane 14, negative control; lane 15, positive fungal DNA; lane 16, positive internal standard DNA, (b) relationship between fungal DNA and PCR product ratio.

CHAPTER 3

Effect of eyespot caused by *Oculimacula yallundae* and *O. acuformis,* assessed visually and by quantitative PCR, on yield and lodging resistance of winter wheat

INTRODUCTION

Yield losses due to eyespot disease on winter wheat are associated with direct effects of moderate or severe lesions, interfering with the movement of nutrients and water through the stem and indirect effects of severe lesions causing lodging (Glynne & Salt, 1958; Scott & Hollins, 1974; Clarkson, 1981). Winter wheat grain losses of up to 33 % in the absence of lodging have been reported following artificial inoculation (Oort, 1936; Glynne & Salt, 1958). Moderate or severe eyespot lesions have been shown to reduce thousand grain weight by up to 5% and 15%, respectively (Defosse & Rixhon, 1968; Scott & Hollins, 1974; Clarkson, 1981). The ultimate effect of severe eyespot lesions, however, is the weakening of the stems to such an extent that they collapse in all directions (Butler, 1957). In comparison, stem or root lodging caused by natural forces occurs in a uniform direction across the field (Pinthus, 1973).

Scott & Hollins (1978) demonstrated that the percentage yield loss due to eyespot was related more closely to the amount of lodging caused by the disease than to the incidence of severe eyespot. Conversely, the occurrence of lodging has been linked to high incidence of eyespot disease. Following a survey of 167 fields of winter wheat in the UK, Glynne (1942) reported that the percentage area lodged increased with the percentage infection by eyespot and the largest area lodged, more than 15%, was observed in the group of fields showing more than 70% incidence of eyespot at harvest. Many of the cultural field practices as well as environmental conditions conducive to natural lodging are also conducive to eyespot disease. For example, early sowing has been shown to favour both eyespot (Colbach & Saur, 1998) and natural lodging (Pinthus, 1973). Abundant moisture supply may increase not only lodging risk but also the incidence and severity of eyespot (Pinthus, 1973). Therefore, it is unclear if the correlation between

eyespot disease and lodging is because of positive effects of eyespot on lodging or the effects of certain plant characteristics or environmental factors influencing both.

Crook & Ennos (1994) investigated the mechanical basis of stem lodging in winter wheat cultivars and determined that their resistance to simulated lodging was related more to the stem characteristics of the plants than to the bending strength or rigidity of the stem. Natural stem lodging occurred during grain filling when wheat ears were heaviest and the stem failed to support the overturning moment generated by the "self-weight" of the plant (stem height and weight, including the ear). Thus, lodging resistance was associated with shorter and lighter plant shoots. The outcome of severe eyespot on winter wheat, however, is the reduction of ear weight (Scott & Hollins, 1974). Severely infected plants would therefore be expected to have much lighter shoots than uninfected plants. In these terms, it is difficult to identify the exact mechanism of eyespot-induced lodging, since the lodging resistance of eyespot infected plants would be expected to be higher, because of reduced stem weight, due to direct effects of the disease. Still, eyespot-induced lodging has been shown to occur in such lighter crops, severely infected by the disease. Indeed, Glynne (1958) reported that an increase in stem weight from 5 to 7.5 t ha^{-1} had relatively similar effect of increasing lodging as an increase from 35 to 85% of stems with severe eyespot. Therefore, this study will attempt to quantify and identify the exact mechanisms and relative importance of plant characteristic against stem bending strength for eyespot-induced lodging risk.

Cultivar improvements may partially explain why natural lodging is not a common occurrence at present. Modern cultivars have been bred to have shorter stems capable of resisting natural forces to which they are subjected. However, with the discovery that eyespot disease is caused by two separate species, it is unclear from previous research if both species are capable of causing severe disease to the extent that they induce lodging of modern cultivars. It is also uncertain if the effects of eyespot on yield of winter wheat as determined by Scott & Hollins (1974) and Clarkson (1981) are the same for both

Oculimacula spp., since at the time of their work, *O. yallundae* was the predominant pathogen, causing the disease in the UK.

The aims and objectives of this chapter are to examine the relationships between pathogen DNA, disease severity and yield loss in winter wheat caused by *O. yallundae* or *O. acuformis* and to relate plant characteristics, associated with lodging resistance, to eyespot disease and DNA of each *Oculimacula* spp., quantified using PCR assays.

Null hypotheses tested: Eyespot has no effect on lodging resistance of infected plants. The effects of *O. yallundae* and *O. acuformis* on those plant characteristics associated with lodging resistance are the same. Severe eyespot caused by *O. yallundae* is likely to cause the same yield loss as severe eyespot caused by *O. acuformis*.

MATERIALS AND METHODS

Field operations and disease assessment

Winter wheat cultivar Consort was grown following a break from cereals of more than nine years, immediately preceding crops were potatoes and carrots. The field site in Tibberton, Shropshire was chosen because of the long period of break from cereal production thus minimising the risk of naturally occurring eyespot. All field operations carried out on the site are listed in Appendix 7. The experiment was set up as a randomised block design with four 1.8x12 m replicated plots for each treatment. Plots were artificially inoculated with three individual isolates of *O. yallundae* or *O. acuformis* or left untreated (control). A guard strip of 0.5 m between each plot was left in order to reduce the movement of inoculum between plots. To promote conditions conducive to lodging, growth regulators were not applied to the crop. Early in the season poor plant growth up to stem extension was observed due to severe manganese deficiency and weed

infestation on the site. The doses and rates of manganese treatments and herbicides applied on the field are listed in Appendix 7.

Plots were harvested using a small plot combine harvester (Winterstieger Crop Master), and grain yield (at 15% moisture content), thousand grain weight and specific (hectolitre) weight were measured.

Isolates of Oculimacula *spp. used, inoculum preparation and application*

Fungal isolates of *Oculimacula yallundae* and *O. acuformis* were isolated and maintained as described in Chapter 2. Isolates used for inoculation are listed in Table 3.1. Oat-grain inoculum was prepared as described in Chapter 2 and applied manually at a rate of 6 g m^{-2} when the crop was at GS 12. Following inoculation, pathogen DNA present in oat-grain inoculum was extracted from one sample of 10 g of oats from each thoroughly mixed oat-grain inoculum of each isolate. Pathogen DNA was quantified using competitive PCR assays (Table 3.1).

Table 3.1. Details of isolates of *Oculimacula* spp. used in the inoculated experiment and pathogen DNA quantified from oat-grain inoculum, Tibberton 2001.

Isolate	Species	Pathogen DNA pg ng^{-1} of total DNA
136/8	*O. yallundae*	4.8
109/13	*O. yallundae*	0.7
166/3	*O. yallundae*	2.9
130/4	*O. acuformis*	2.2
159/7	*O. acuformis*	0.9
162/3	*O. acuformis*	0.8

Sampling and disease assessment

Disease assessments for eyespot were carried out on 30 plants per plot collected at GS 33 and on 30 main shoots at GS 60 from each plot as described in Chapter 2. Immediately following assessment, pathogen DNA was extracted from plant material and quantified using competitive PCR as described in Chapter 2. At GS 33, single shoots and tillers of five plants per plot were counted, weighed and assessed for eyespot on a scale of 0 to 3, where 0 was not infected and 3 was severely infected based on Scott & Hollins (1974) as described in Chapter 2. Then, a disease index was calculated (Scott & Hollins, 1974) for each plant and averaged for each plot.

Single plant shoots at GS 72 and assessment of lodging resistance

Fifteen plants at GS 72 were collected at random from each plot. The main shoot from each plant was separated manually from the tillers and kept in a plastic bag in a cold room at 4°C to minimise loss of fresh weight and pathogen DNA accumulation. Assessments of all shoots (total of 900 single main shoots) were made within 72 h of sampling. Height, fresh weight, and centre of gravity were recorded for each shoot (Crook & Ennos, 1994). The centre of gravity was determined by placing each shoot across an outstretched index finger and moving the shoot along the finger until the balance point was reached. The height of the centre of gravity was the distance from the base of the stem to the balance point. The ear of each shoot was removed and weighed and the shoot was then assessed for disease severity (Scott & Hollins, 1974).

Mechanical tests on stems

Stem lodging occurs naturally when the stem cannot support the overturning moments generated by the self-weight of the plant (Crook & Ennos, 2000). The lodging meter used in this study (Appendix 8) measures the bending strength of the stem. A simplified diagram representing the process of natural lodging can be seen in Appendix 9.

The basal 7 cm length of each shoot was cut and placed (eyespot lesions always facing the same direction) in the holding cup (1 cm deep) of the lodging meter and the lodging arm was rotated until the stem buckled (Appendix 8, Crook & Ennos, 2000). Failure occurred at the eyespot lesion which was usually at 2-4 cm from the base of the stem. The maximum force required to buckle the stem, thus bending strength (Nm), was recorded for each shoot. The self-weight moment (M, Nm) of each main shoot at 30° from the vertical was calculated:

$M = sin\ 30^{\circ} \times h \times mg$

Where h is the height to the centre of gravity for each shoot from the ground (m) and m is the mass of each shoot (g), g is the acceleration due to gravity (9.81 ms^{-2}) (Crook & Ennos, 1994). Safety factors against stem lodging were calculated by dividing the bending strength (Nm) of the main shoot by the self-weight moment generated by that shoot (Nm) (Crook & Ennos, 1994).

DNA extraction and quantification of single shoots

Following mechanical tests, the same stem cuttings were chopped finely and freeze dried for DNA extraction. Each shoot was placed in a 57x15.3 mm length plastic tube (Sarstedt Ltd, Leicester, UK) together with three steel ball bearings (8 mm diameter) and homogenised using a soil mill for 1-2 h. DNA extraction was carried out as described in Chapter 2, except that all ingredients were adjusted for the smaller volume of plant

material. Total DNA concentration was determined and quantitative PCR performed on DNA extracted from each shoot as described in Chapter 2.

Statistical analysis

All data were analysed using analysis of variance and regression analysis using Genstat® Version 4.1 for Windows (Lawes Agricultural Trust, UK). Where necessary, DNA and incidence data were transformed using log_{10} and angular transformations, respectively, in order to normalise the data's distributions. Data from single shoot assessments were separated and averaged according to eyespot score of slight, moderate and severe for each treatment. Multiple linear regressions with groups were used for to analyse relationships between variables for the two *Oculimacula* spp.

RESULTS

Disease incidence, severity and DNA accumulation for O. acuformis *and* O. yallundae

No significant differences were observed between isolates within species and all caused eyespot disease with similar incidence or severity at each growth stage. At GS 33, plants inoculated with isolates of *O. yallundae* had significantly higher eyespot incidence and DI (assessed using 30 plants or 5 plants and tillers), than plants inoculated with isolates of *O. acuformis* or the control (Table 3.2). The incidence of eyespot at GS 33 was 35.2 and 68.9 % for plots inoculated with *O. acuformis* and *O. yallundae*, respectively. At GS 60 and 72 no significant differences were observed between plants inoculated with *O. acuformis* or *O. yallundae*, but the control plants had significantly lower incidence and DI than inoculated plants with either species.

DNA concentrations measured at each growth stage are presented in Table 3.3a&b. PCR quantification revealed that DNA of both species was present as early as GS 33 in the control (1.10 and 0.23 pg $ng^{-1}$of total DNA for *O. yallundae* and *O. acuformis*, respectively). No significant difference was found between isolates within each species for DNA concentrations measured from bulk extractions at GS 33 and 60 (Table 3.3a). Pathogen DNA in the control plants increased at GS 60 and GS 72 to 4.1 and 31.7 pg ng^{-1} for *O. yallundae*, and 8.1 and 9.0 pg ng^{-1} for *O. acuformis*, respectively (Table 3.3b). At GS 33, plants inoculated with *O. acuformis* had nearly 50 % less pathogen DNA present in their stems than those inoculated with *O. yallundae*. By GS 60, pathogen DNA in the plants inoculated with *O. acuformis* was significantly higher than in those inoculated with *O. yallundae*, in which concentrations of *O. yallundae* DNA were similar to DNA concentrations of both species found in the control plots. The reverse result was obtained for DNA quantified from single stem extractions at GS 72, where *O. yallundae* was found in five times higher concentrations than *O. acuformis*. Total (plant and fungal) DNA quantification of single shoot DNA at GS 72 revealed that shoots inoculated with *O. yallundae* had 25-50% lower total DNA concentrations than shoots inoculated with *O. acuformis*. Indeed, it was noted during eyespot assessments at GS 72 that many of the main shoots, infected with *O. yallundae* had much more straw-like appearance compared to shoots infected with *O. acuformis* or the control.

The increased proportion of fungal to plant DNA, which is often associated with decreases of plant DNA during plant maturation and/or senescence, may partially explain the large differences between DNA concentrations of *O. yallundae* quantified at GS 60 from bulk plant material and GS 72 from individual main shoots.

Table 3.2. Mean incidence and DI for eyespot caused by *O. yallundae* and *O. acuformis* on winter wheat.

	GS 33			GS 60[c]		GS 72[d]	
Inoculated	I[a]	DI[a]	DI[b]	I	DI	I	DI
treatment			%*				
Control	21.9(13.9)	20.8(12.6)	22.8(15.0)	85.1(99.3)	56.5(69.5)	65.5(82.8)	43.7(47.7)
O. y[e]	56.1(68.9)	52.7(63.3)	53.5(64.6)	87.2(99.8)	76.2(94.3)	84.8(99.0)	66.9(84.6)
O. a[f]	36.4(35.2)	33.0(29.7)	38.6(38.9)	89.6(99.9)	70.9(89.3)	80.9(97.5)	60.0(75.0)
SED	3.82	3.63	4.75	2.4	3.43	3.40	3.12
CV	26.1	26.7	32.4	7.9	13.8	12.1	14.8
LSD	7.7	7.3	9.5	4.9	6.9	6.8	6.3
P	< 0.001	< 0.001	< 0.001	0.178	< 0.001	< 0.001	<0.001

[a] assessments made on thirty plants per plot, [b] assessments made on main shoot and tillers of five plants per plot, [c] assessments made on the main shoot of thirty plants per plot, [d] assessments made on the main shoot of fifteen plants per plot, [e] plots inoculated with *O. yallundae*, [f] plots inoculated with *O. acuformis*
* angular transformed data, back-transformed means in parentheses

Table 3.3a. Mean DNA of *O. yallundae* and *O. acuformis* on winter wheat.

	log_{10} of pathogen DNA pg ng^{-1} of total DNA*		
Inoculation	GS 33[a]	GS 60[b]	GS 72[c]
treatment			
Control	-	-	-
O. y	0.81(6.40)	1.10(12.53)	2.30(197.69)
O. a	0.53(3.37)	1.60(39.90)	1.50(31.33)
SED	0.10	0.10	0.08
CV	53.7	26.6	13.9
LSD	0.21	0.21	0.15
P	0.010	< 0.001	< 0.001

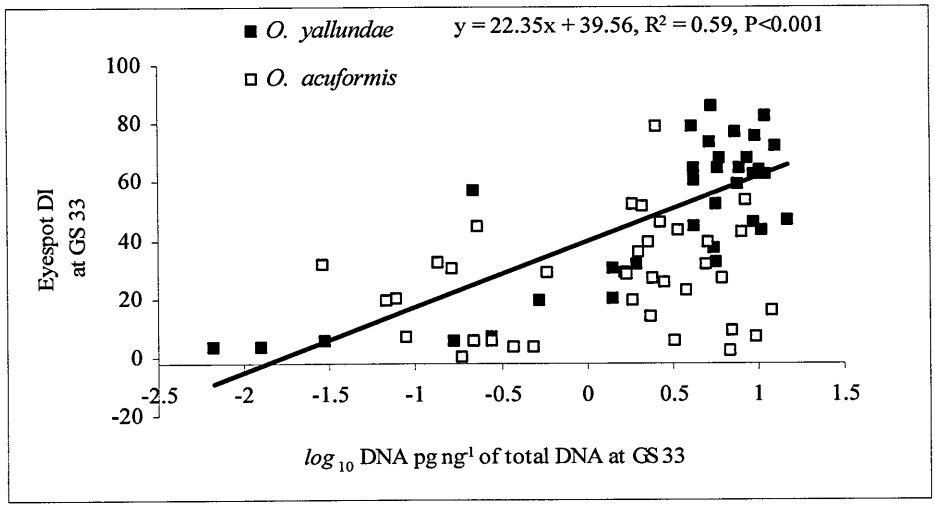
* Back-transformed means in parentheses, [a] bulk DNA extraction from thirty plants per plot, [b] bulk DNA extraction from the main shoot of thirty plants per plot, [c] average for DNA extraction from individual main shoots of fifteen plants per plot

Table 3.3b. Mean background DNA of *O. yallundae* and *O. acuformis* on winter wheat (data not transformed).

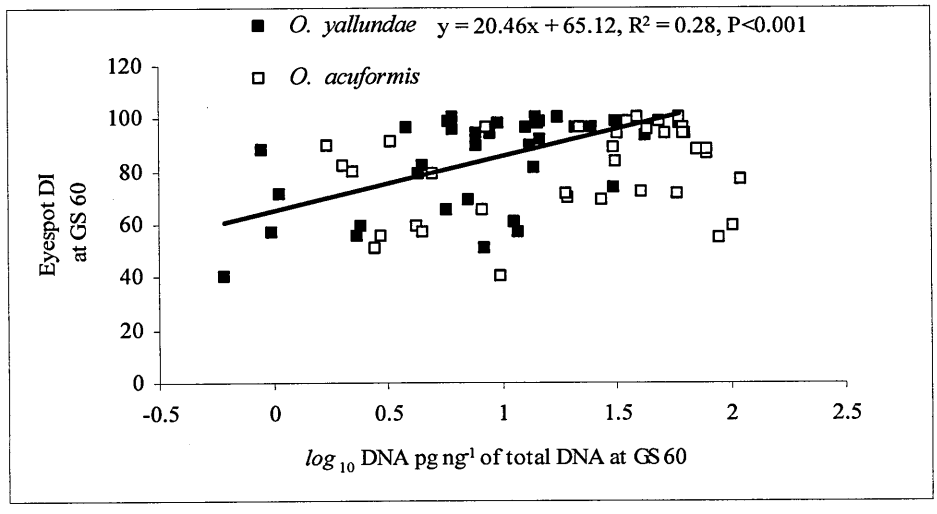
	Pathogen DNA pg ng^{-1} of total DNA		
Inoculation	GS 33[a]	GS 60[b]	GS 72[c]
treatment			
Control[d]	1.33	12.19	40.8
O. y[e]	0.72	4.8	0.2
O. a[e]	0.84	1.0	5.1
SED	0.78	1.59	5.5

[a] bulk DNA extraction from thirty plants per plot, [b] bulk DNA extraction from the main shoot of thirty plants per plot, [c] average for DNA extraction from individual main shoots of fifteen plants per plot, [d] DNA of both species present at each growth stage, [e] mean background DNA of the other species (*O. acuformis* DNA found in plots inoculated with *O. yallundae* and vice versa).

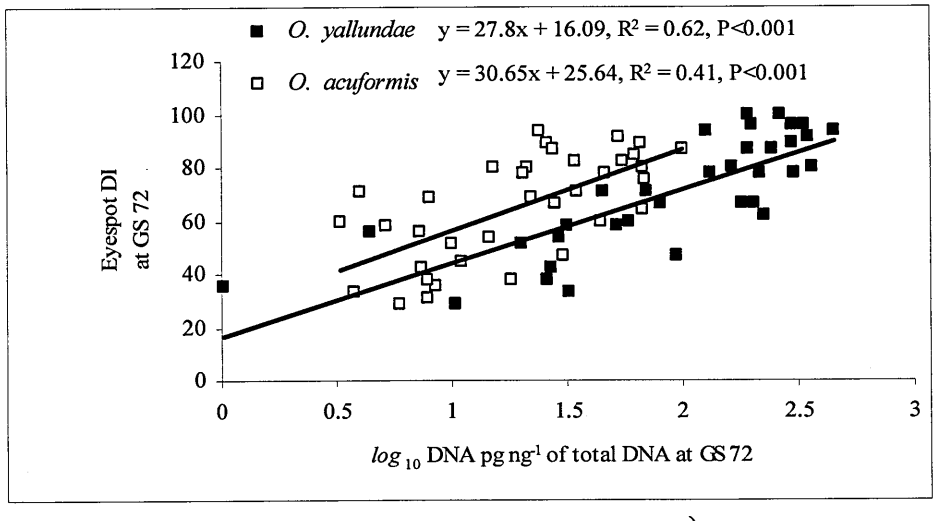
Regressions of disease index on pathogen DNA at the same growth stage were always significant, accounting for 65% of variance for disease symptoms caused by *O. yallundae* (Fig. 3.1). Although both species fitted the same line, the regression was not significant at GS 33 and GS 60 when *O. acuformis* caused the disease (Fig. 3.1, regression equations not shown). Both species also fitted the same regression line when using data from visual assessments at GS 33 to predict disease index at GS 60 or GS 72 (Figures 3.2a & b, 3.3a & b) and relationships, although significant, were weak possibly because of the lack of correlation for plots inoculated with *O. acuformis*. By using data from plots inoculated with *O. yallundae* only, the regression analysis showed much stronger correlation between DI at GS 33 and 60 or 72 (35d.f, $P<0.001$, $R^2=0.43$ and 0.58, respectively). Stronger relationships were observed between DNA at GS 33 and eyespot DI at GS 60 and 72 and, again, both species fitted on the same line (Fig.3.2c & 3.3c). However, the correlations between eyespot DI at GS 33 and pathogen DNA at GS 60 or 72 were stronger for *O. yallundae* than for *O. acuformis* (Fig.3.4a & b, 3.5a & b) consistent with eyespot symptoms caused by this pathogen correlating better with its DNA concentration at each growth stage (Fig 3.1). Regressions of pathogen DNA at GS 60 and 72 on DNA at GS 33 were highly significant for both species (Fig. 3.4c & 3.5c). Stronger relationships between early and late eyespot disease for *O. acuformis* were found when regressing pathogen DNA concentrations (Fig.3.5c, 3.6a & b) than disease incidence or severity at different growth stages.



a)

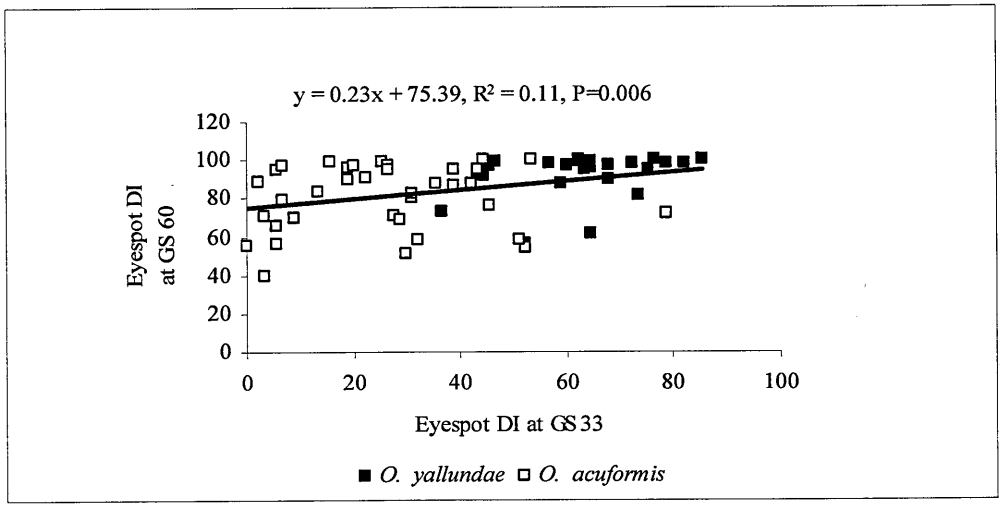


b)

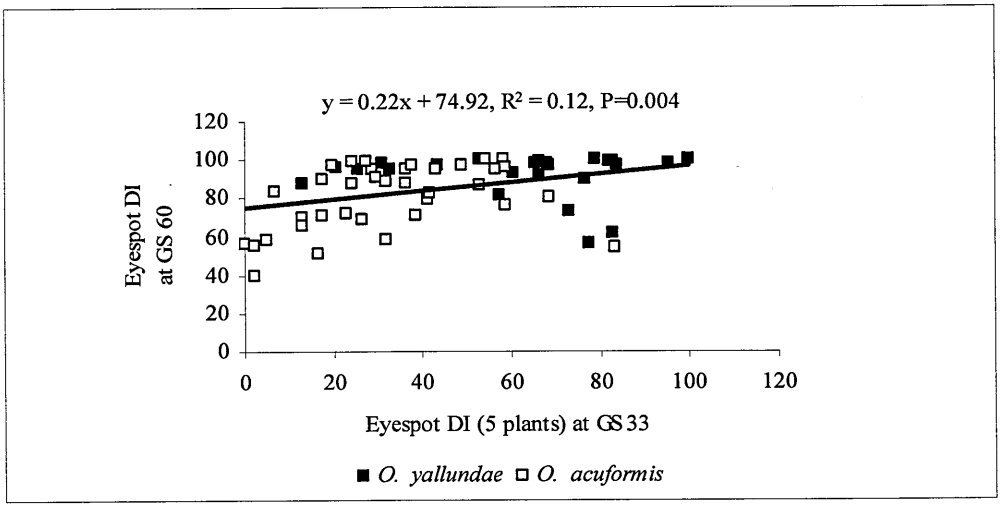


c)

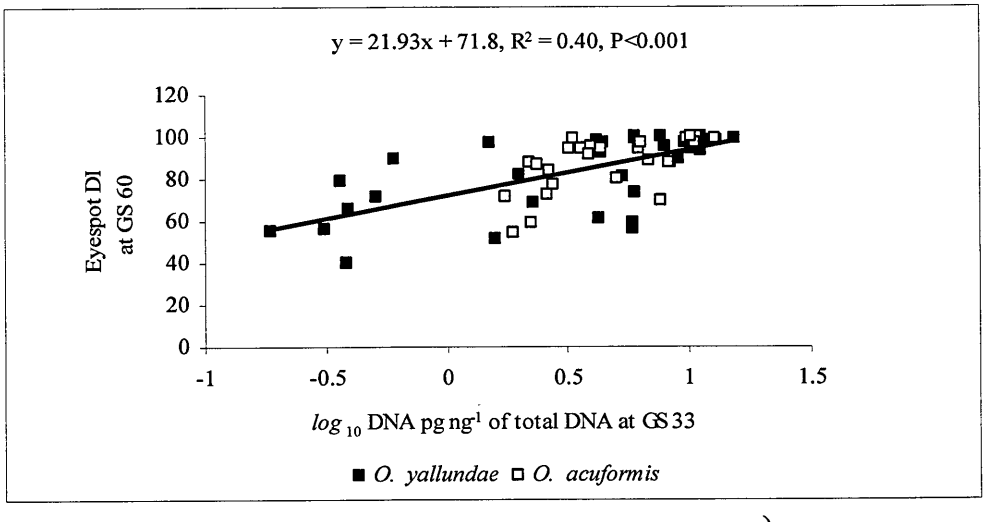
Figure 3.1. Regressions of eyespot DI on pathogen DNA at GS 33 (a), GS 60 (b) and GS 72 (c). Equations shown on Fig. 3.1a&b relate to relationships only for *O. yallundae*.



a)

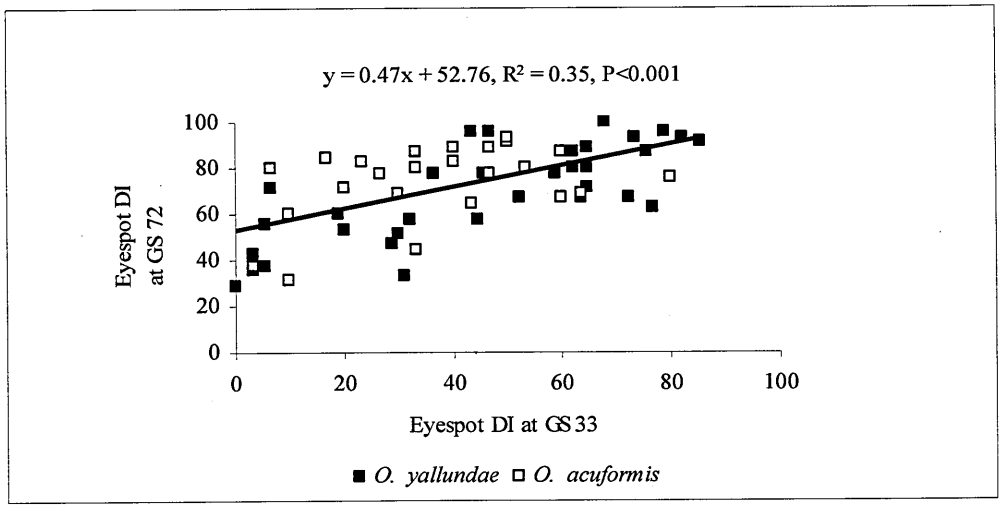


b)

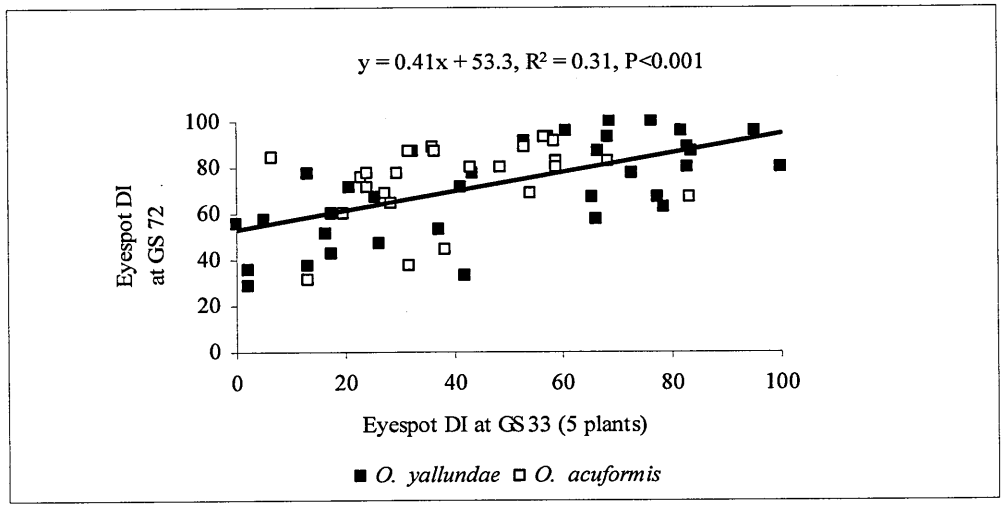


c)

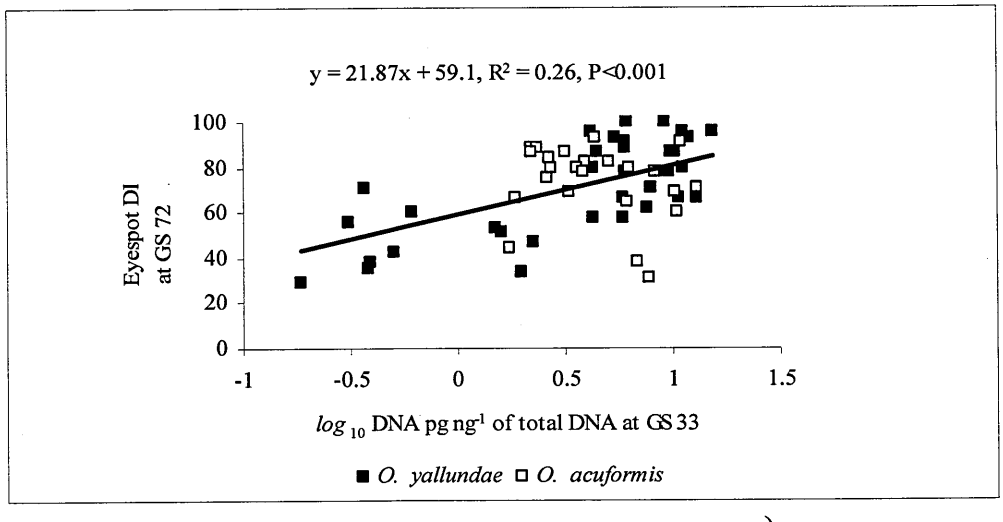
Figure 3.2. Regressions of eyespot DI at GS 60 on eyespot DI at GS 33 (a), GS 33 (5 plants) (b) and DNA of *O. acuformis* and *O. yallundae* at GS 33 (c).



a)

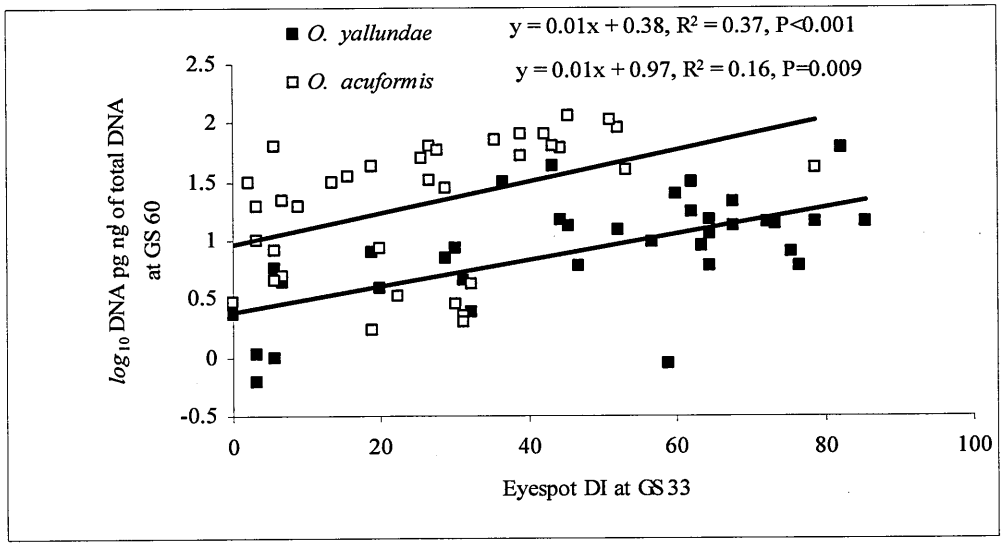


b)

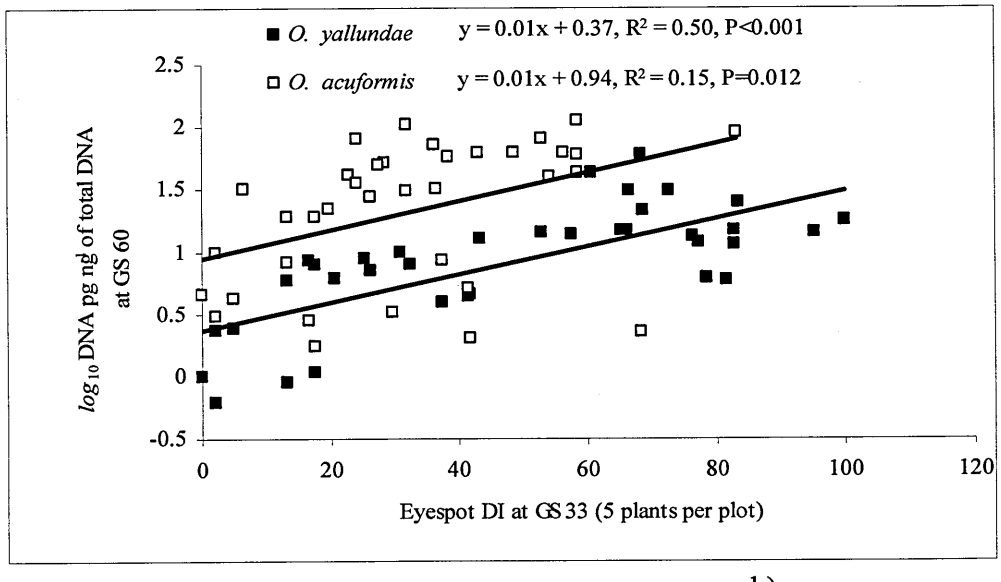


c)

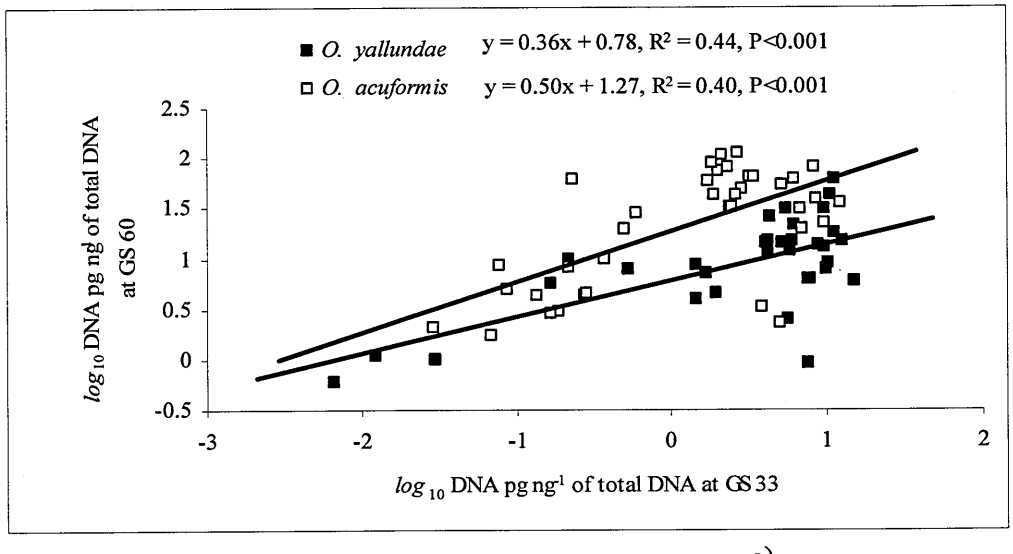
Figure 3.3. Regressions of eyespot DI at GS 72 on eyespot DI at GS 33 (a), eyespot DI at GS 33 (5 plants) (b) and DNA of *O. acuformis* and *O. yallundae* at GS 33 (c).



a)

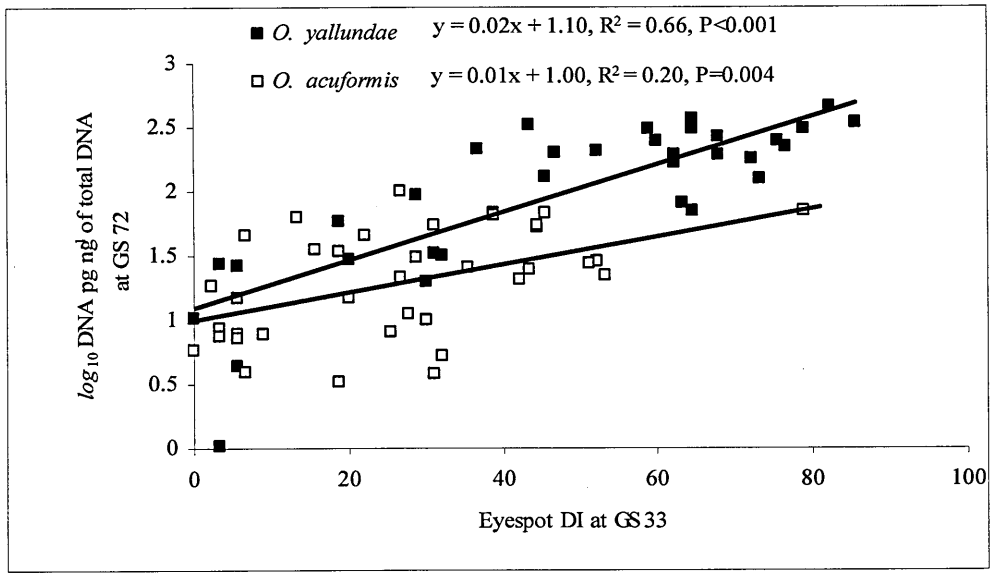


b)

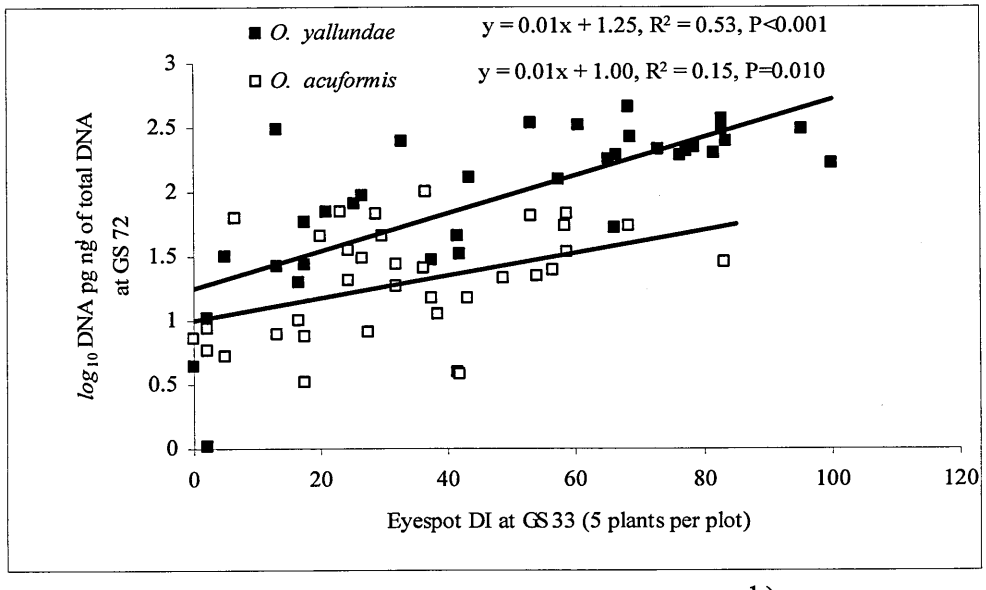


c)

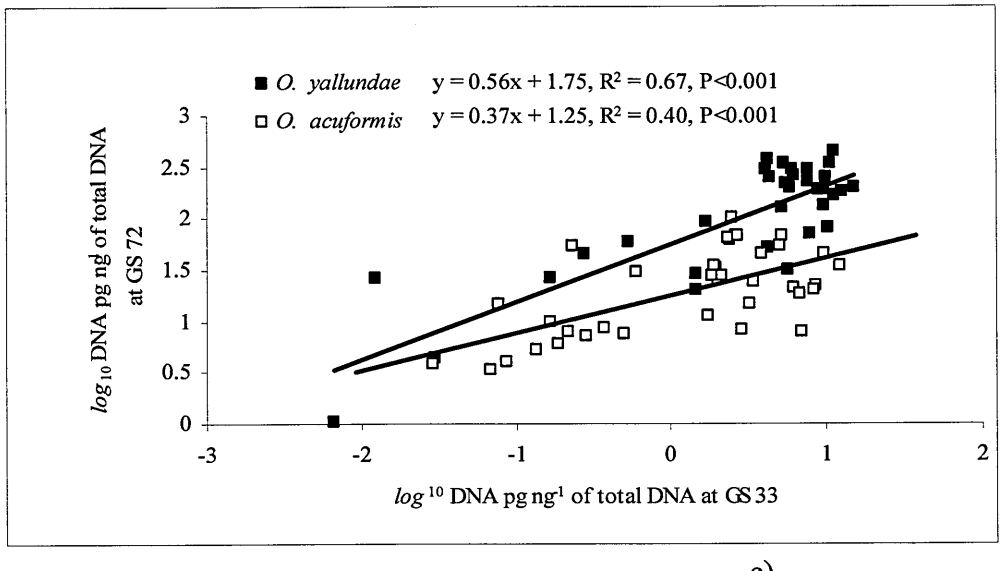
Figure 3.4. Regressions of DNA of *O. acuformis* and *O. yallundae* at GS 60 on eyespot DI at GS 33 (a), eyespot DI at GS 33 (5 plants) (b) and pathogen DNA at GS 33 (c).



a)

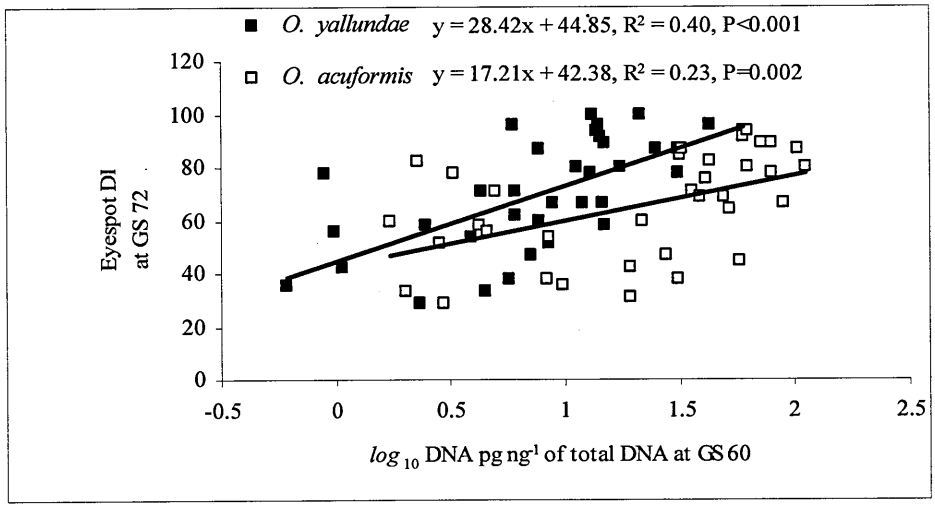


b)

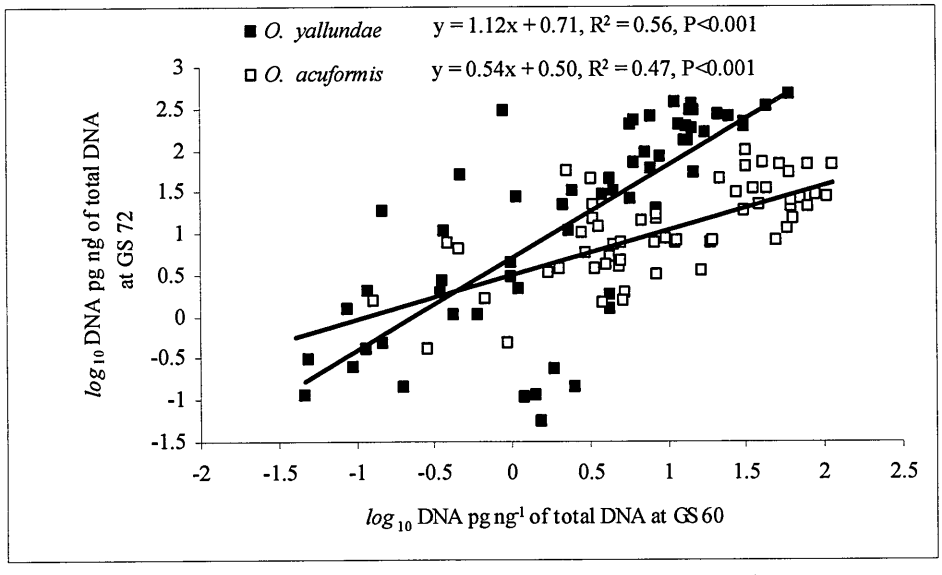


c)

Figure 3.5. Regressions of DNA of *O. acuformis* and *O. yallundae* at GS 72 on eyespot DI at GS 33 (a), eyespot DI at GS 33 (5 plants) (b) and pathogen DNA at GS 33 (c).



a)



b)

Figure 3.6. Regressions of eyespot DI at GS 72 (a) and pathogen DNA at GS 72 (b) on DNA of *O. acuformis* and *O. yallundae* at GS 60.

Lodging failed to occur at the field site. No significant differences were observed between plots inoculated with the two *Oculimacula* species for any of the plant characteristics or yield measured in this study. Eyespot caused by either species had no significant effect on fresh weight of tillers at GS 33 (4.01, 4.05 and 3.86 g for the control, *O. yallundae*-inoculated and *O. acuformis*-inoculated plants, respectively). A significant reduction in tiller number at GS 33 was observed for inoculated plants with either species compared to the control (Table 3.4). At GS 72, no significant effects on tiller height, ear weight or centre of gravity averaged per plot were observed (Table 3.4). However, it must be noted that by GS 72, high concentrations of DNA of both species were found in the control plots (Table 3.3), thus the comparison was limited due to the lack of disease free plants. Even under such circumstances, significant differences between inoculated and non-inoculated plots were found for stem bending strength, safety factor, and yield. Stems with moderate lesions contained 27 and 157 pg ng^{-1} DNA of *O. acuformis* and *O. yallundae* and reduced stem safety factor by 13 and 14% compared to the control, respectively (Table 3.5). Severe lesions corresponded to 40 and 234 pg ng^{-1} DNA of *O. acuformis* and *O. yallundae* and caused reductions of stem safety factor of 36 and 33%, respectively. Based on averaged values per plot, only *O. yallundae* reduced stem bending strength significantly compared to the control plants (Table 3.4). Although, slight and moderate lesions of both species failed to show an effect on stem strength, severe lesions reduced stem bending strength by 35%. Eyespot caused by either species had no effect on ear weight as indicated by the analysis of averaged values per plot (Table 3.4). At harvest, plants inoculated with *O. acuformis* had significantly lower grain yield than the control. *Oculimacula acuformis* reduced overall yield by 11%. Although smaller yields were also measured from plots inoculated with *O. yallundae*, overall yield was not significantly different from the control.

Significant differences were observed between isolates of *O. acuformis* for DNA concentrations at GS 72, tiller weight and self-weight moment (Table 3.6a) and between isolates of *O. yallundae* for background DNA of *O. acuformis*, bending strength and safety factor (Table 3.6b). Isolates of *Oculimacula acuformis* with lower DNA concentration at GS 72 caused less reduction in tiller weight and self-weight moment. Isolates of *O. yallundae* showed greater effect on stem bending strength and safety factor where lower concentrations of background DNA of *O. acuformis* were found.

Table 3.4. Plant stem characteristics, bending strength, safety factor and yield means for plots inoculated with *O. yallundae*, *O. acuformis* and the control.

	GS 33					GS 72					
Inoculation treatment	Tiller number	Tiller height cm	Tiller weight g	Centre of gravity cm	Self-weight moment Nm	Bending strength Nm	Safety factor M	Ear weight g	Yield t ha^{-1}	TGW g	Specific weight hl
Control	2.47	81.35	7.52	46.35	0.018	0.126	6.98	4.12	8.18	42.88	73.78
O. y	2.19	79.97	7.25	45.69	0.017	0.104	5.99	4.07	7.64	41.81	73.49
O. a	2.14	80.75	7.58	46.01	0.018	0.112	6.17	4.20	7.26	41.01	72.60
SED	0.13	0.85	0.27	0.56	0.0007	0.008	0.40	0.125	0.35	0.85	0.53
CV	16.8	3.0	10.1	3.5	11.1	20.0	17.8	8.5	13.3	5.8	2.1
LSD	0.26	1.70	0.53	1.13	0.001	0.016	0.79	0.25	0.71	1.71	1.07
P	0.048	0.23	0.28	0.48	0.50	0.025	0.045	0.43	0.042	0.09	0.051

Table 3.5. Pathogen DNA concentration and effects of moderate and severe eyespot caused by *O. yallundae and O acuformis* on stem safety factor and stem bending strength at GS 72.

Inoculation treatment	*log* $_{10}$ pathogen DNA* pg ng^{-1} of total DNA		Stem safety factor M		Bending strength Nm	
	ME[a]	SE[b]	ME	SE	ME	SE
Control	0.90(7.90)	0.90(7.9)	7.65	7.63	0.137	0.137
O. y	2.20(157.8)	2.38(234.4)	6.60	5.14	0.120	0.085
O. a	1.43(27.0)	1.62(40.7)	6.67	4.91	0.126	0.085
SED	0.15	0.14	0.40	0.35	0.008	0.007
CV	33.8	29.1	19.1	20.2	20.1	24.2
LSD	0.30	0.28	0.79	0.71	0.016	0.015
P	<0.001	<0.001	0.016	<0.001	0.103	<0.001

* back-transformed values are shown in parentheses

[a] moderate eyespot

[b] severe eyespot

Table 3.6a. Effects of different isolates of *O. acuformis* on tiller weight, self-weight moment and DNA concentration at GS 72.

O. acuformis Isolate	Tiller weight g	log_{10} DNA of *O. acuformis*	self-weight moment Nm
130/4	8.29	1.30 (20.04)	0.01955
159/7	7.16	1.53 (33.65)	0.01680
162/3	7.30	1.66 (45.60)	0.01732
SED	0.38	0.13	0.001
CV	10.0	17.8	11.5
LSD	0.80	0.28	0.002
P	0.016	0.046	0.035

Table 3.6b. Effects of different isolates of *O. yallundae* on stem bending strength, safety factor and DNA concentration of *O. acuformis* at GS 72.

O. yallundae Isolate	log_{10} DNA of *O. acuformis*	Bending strength Nm	Safety factor M
109/13	0.64 (4.38)	0.114	6.53
136/8 -	0.93 (8.43)	0.106	6.22
166/4	0.33 (2.13)	0.091	5.24
SED	0.13	0.01	0.47
CV	40.2	16.5	15.6
LSD	0.27	0.02	0.99
P	< 0.001	0.040	0.035

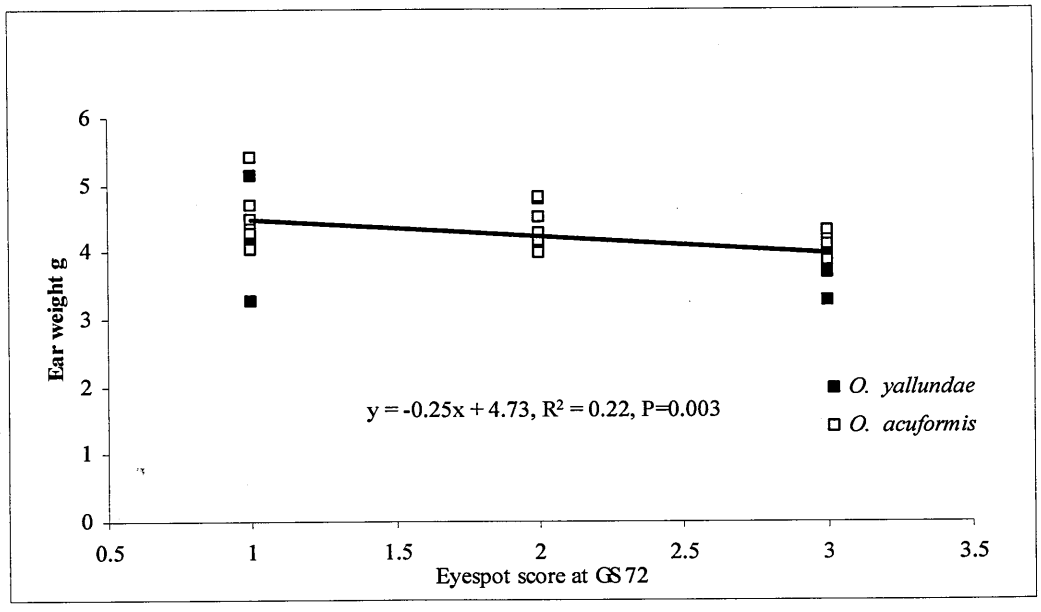
Back-transformed means are shown in parentheses.

Relationships between pathogen DNA, grain yield and plant characteristics associated with lodging resistance

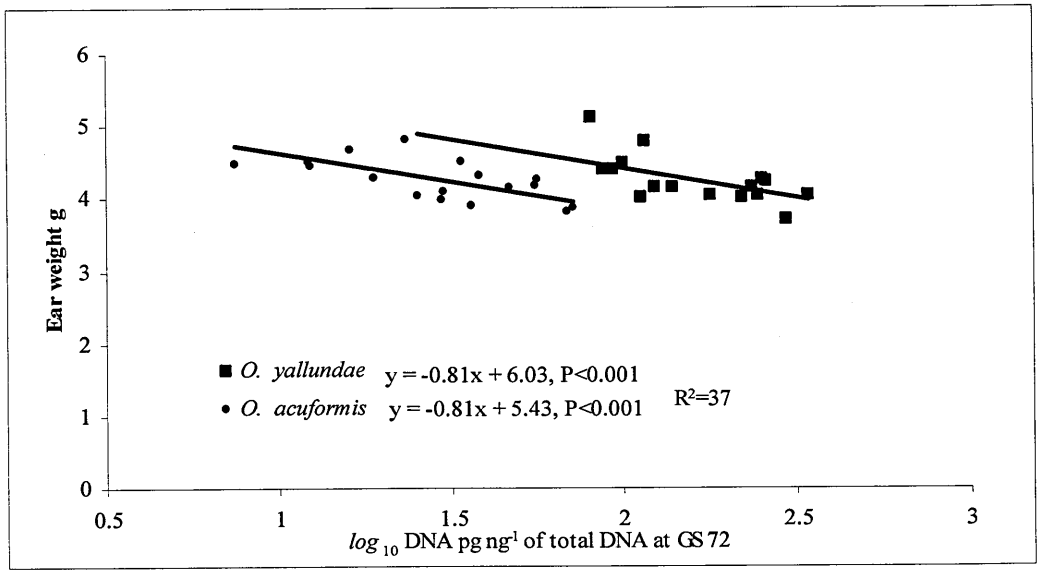
Although a significant negative relationship was observed between DNA of *O. acuformis* at GS 33 and yield, it accounted for only 9 % of the variance (yield (t/ha) = -0.41 x (log_{10} DNA of *O. acuformis* at GS 33) + 7.50, P=0.014, 59 d.f). The same regression analysis performed using DNA of *O. acuformis* at GS 72 found in all main shoots, categorised as slight, moderate and severe revealed a similar relationship (yield (t/ha) = -0.42 x (log_{10} DNA of *O. acuformis* at GS 72) + 7.88, P<0.001, 34 d.f), accounting for 38% of the variance. There were no significant relationships between yield and DNA of *O. yallundae* at any growth stage.

Regressions of plant characteristics associated with lodging on eyespot scores at GS 72 fitted a common line for both *Oculimacula* spp. (Figs. 3.7a-3.11a). However, regressions of those characteristics on DNA concentration of the two fungi fitted two separate lines (Figs. 3.7b-3.11b). The lines were parallel with negative slopes for ear weight (Fig. 3.7b), tiller weight (Fig. 3.8b), self-weight moment (Fig. 3.9b) and safety factor (Fig. 3.10b), indicating that relatively more DNA of *O. yallundae* was required to achieve the same effect as *O. acuformis*. Regression of bending strength on pathogen DNA at GS 72 showed that the species fitted two non-parallel lines, accounting for 61% of the variance (Fig. 3.11b).

The reduction in stem safety factor for all inoculated plots was associated more with reduction in stem bending strength, accounting for more than 85% of variance, than with reduction in self-weight moment of the plants.

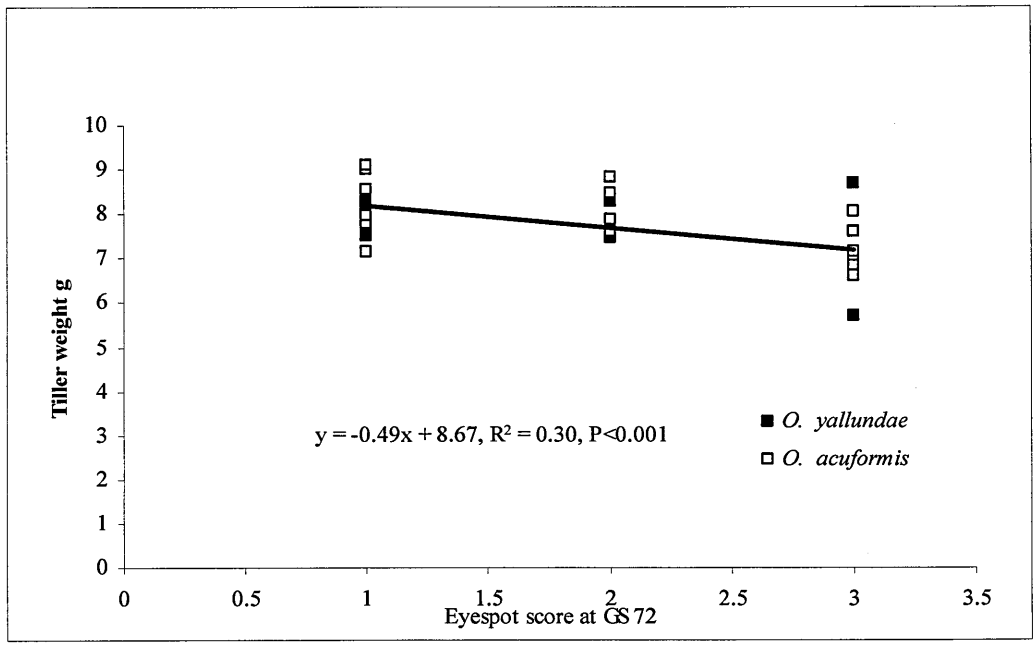


a)

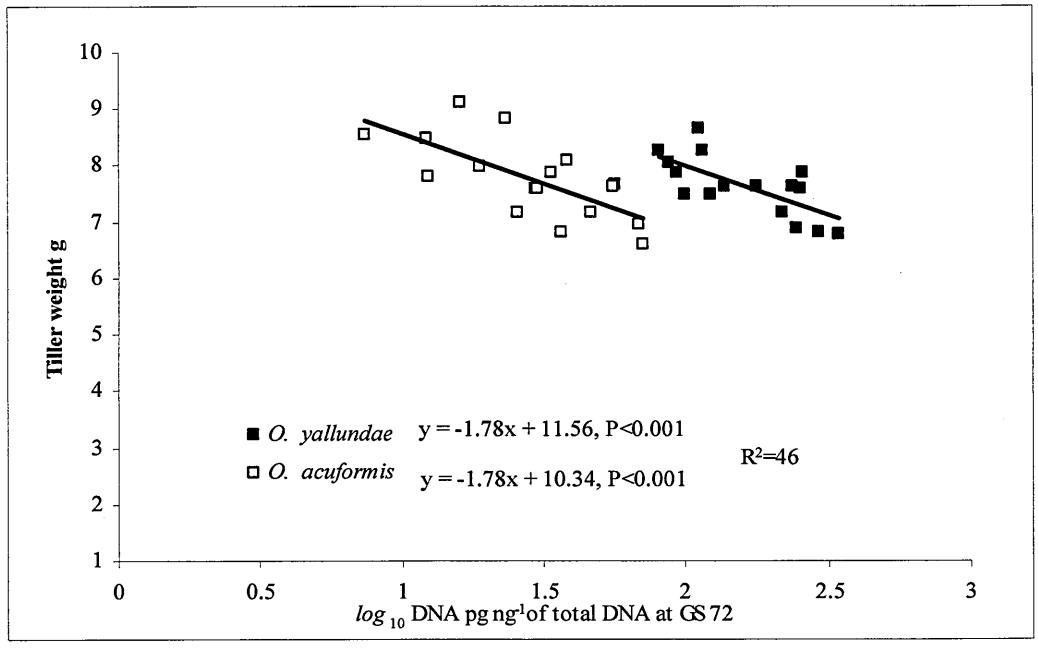


b)

Figure 3.7. Regressions of ear weight on eyespot score at GS 72 (a) and pathogen DNA at GS 72 (b).

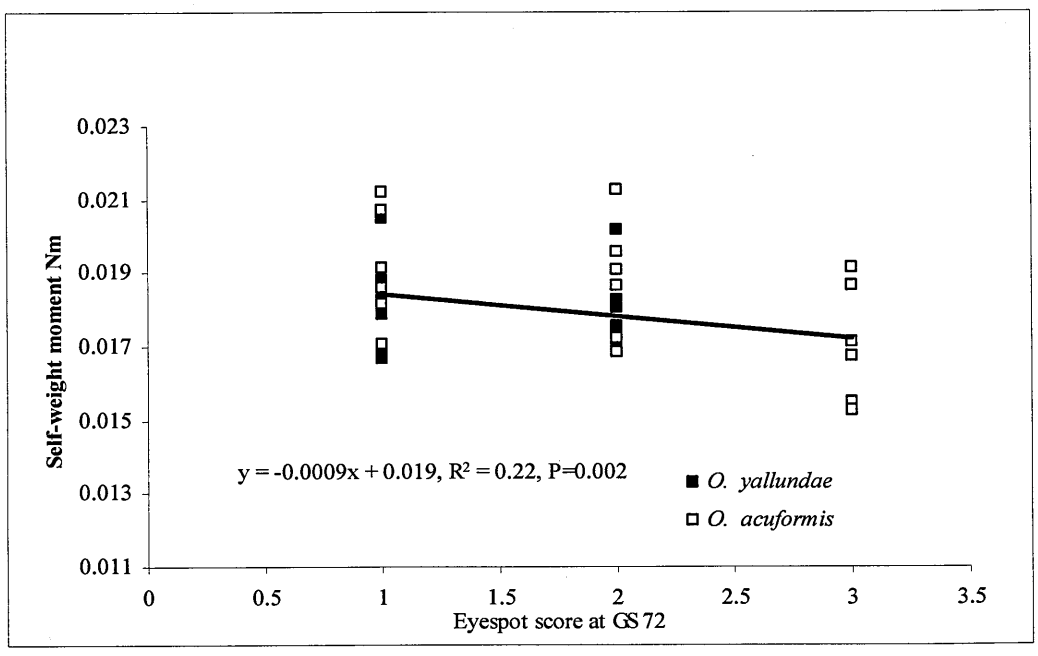


a)

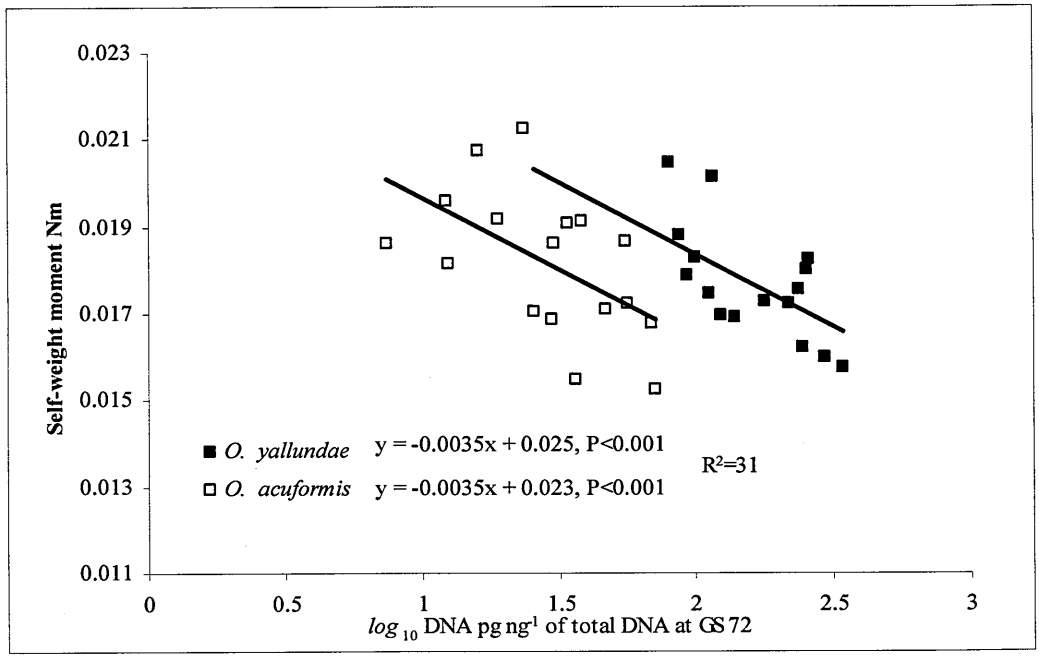


b)

Figure 3.8. Regressions of tiller weight on eyespot score at GS 72 (a) and DNA of *O. acuformis* and *O. yallundae* at GS 72 (b).

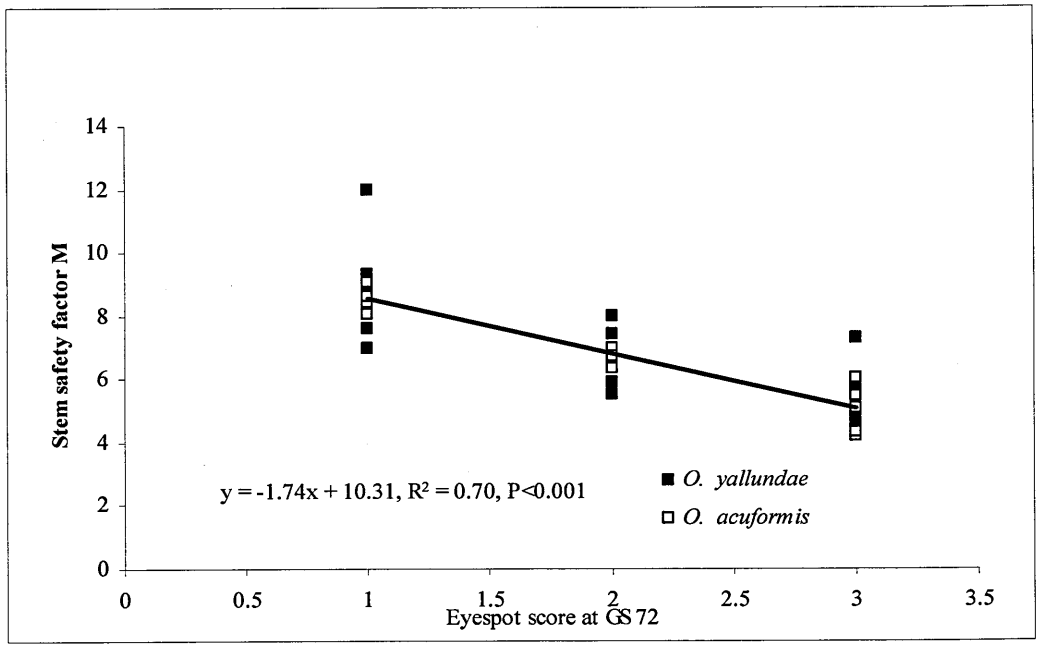


a)

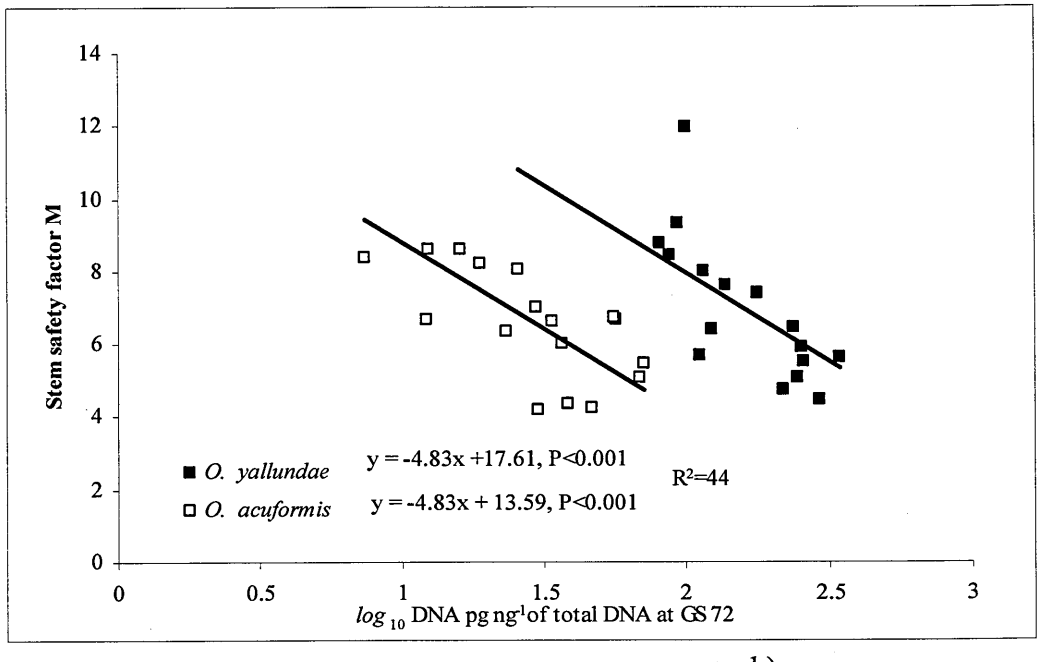


b)

Figure 3.9. Regressions of self-weight moment on eyespot score at GS 72 (a) and DNA of *O. acuformis* and *O. yallundae* at GS 72 (b).

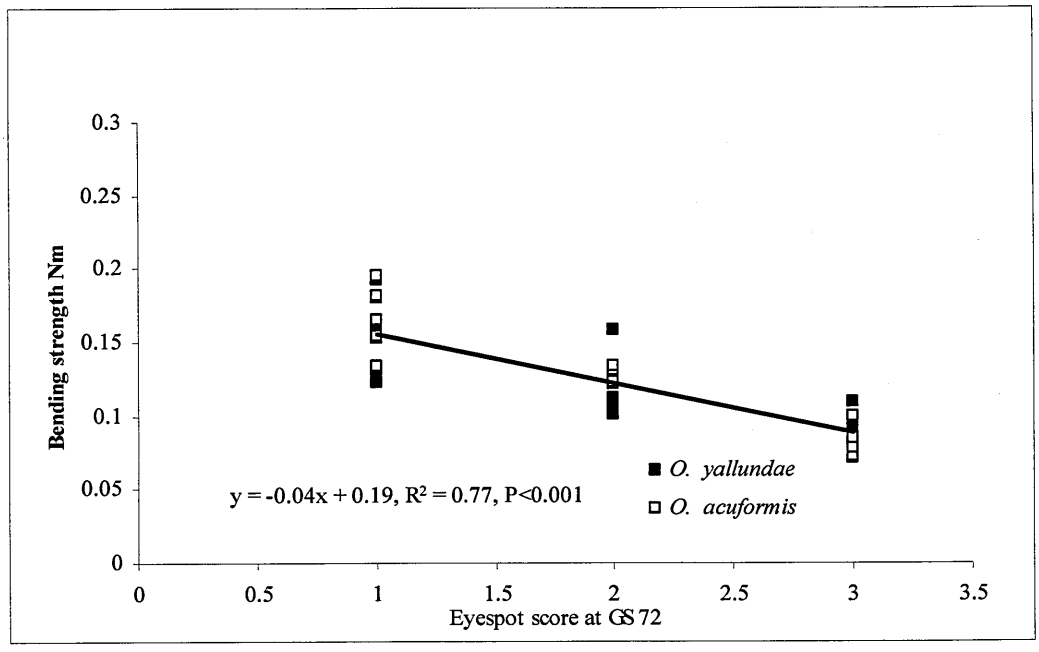


a)

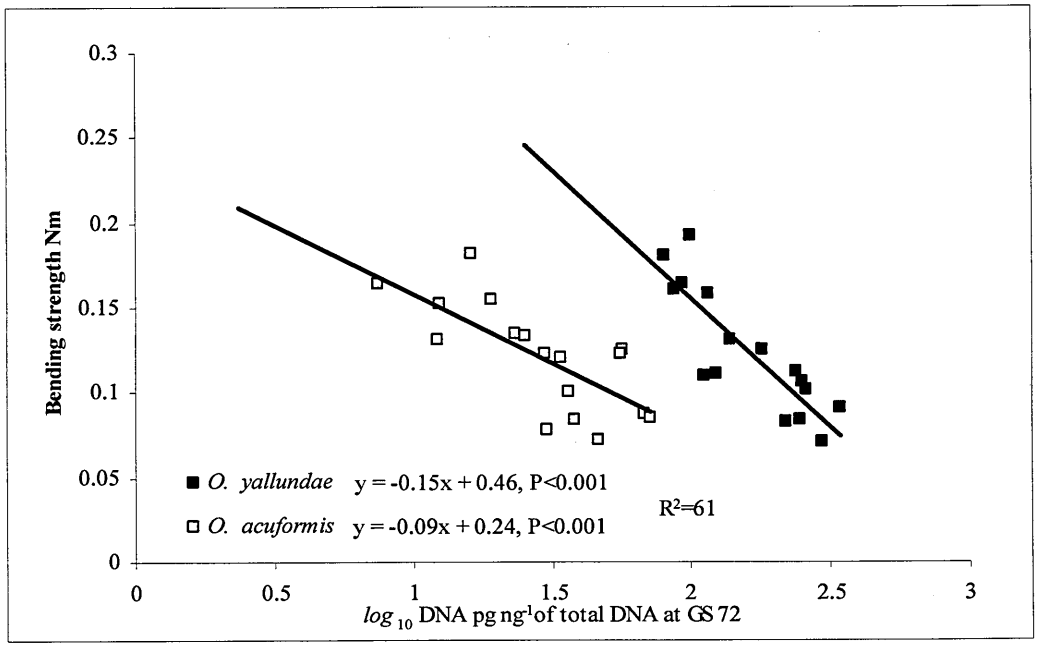


b)

Figure 3.10. Regressions of stem safety factor on eyespot score at GS 72 (a) and DNA of *O. acuformis* and *O. yallundae* at GS 72 (b).



a)



b)

Figure 3.11. Regressions of stem bending strength on eyespot score at GS 72 (a) and DNA of *O. acuformis* and *O. yallundae* at GS 72 (b).

DISCUSSION

The observed differences in eyespot incidence and severity at GS 33 were consistent with previous reports that incidence and severity of eyespot symptoms at the early crop growth stages are greater when the disease is caused by *O. yallundae* (Bateman *et al.*, 1990; Goulds & Fitt, 1991a&b). The higher incidence and DI of plants infected with *O. yallundae* also corresponded to the larger concentrations of pathogen DNA and there was significant positve relationship between DI and quantified DNA at GS 33 (R^2=0.59, P<0.001). In contrast, the symptoms caused by *O. acuformis* failed to correlate with its DNA concentration, indicating that visual diagnosis of eyespot was unreliable for the early stages of the disease when caused by this pathogen. Discrepancies between visual assessment and DNA concentrations of *O. acuformis*, more specifically at the early growth stages of winter wheat, have been reported previously by Turner *et al.* (2001). Such differences could often be explained by the relatively small amounts of *O. acuformis* detected at the time. However, in this study the DNA of *O. acuformis* was quantified to be as much as 4 pg ng^{-1} of total DNA. Therefore, it is likely that such discrepancies may occur because of the differences in symptom expression by the two pathogens at the early crop growth stages, resulting mainly in under estimation of infection when caused by *O. acuformis*. Interestingly, visual assessment using 30 plants or using just 5 plants with all tillers provided similar results, indicating that under high disease pressure sample size is less critical because of more even spread of the disease.

Absence of marked differences between species for disease incidence or severity late in the season has been found in other studies (Goulds & Fitt, 1988). Similarly, in this study, visual assessement failed to determine differences between species at GS 60 and GS 72. However, DNA quantification showed that different concentrations of the two species caused visually similar symptoms. The DNA of *O. acuformis* increased ten-fold from GS

33 to GS 60 and remained at this concentration at GS 72. In comparison, DNA of *O. yallundae* increased only two-fold up to GS 60, but then increased more then ten-fold by GS 72. One reason for this increase at GS 72 could be that, because pathogen DNA is measured against total DNA, and thus decreases in plant DNA are reflected as relative increases in pathogen DNA. Indeed, the plants infected with *O. yallundae* had much more profound straw-like colour associated with lignified, mature tissue and quantification of total DNA showed it to be up to 50% less than total DNA extracted from *O. acuformis* infected plants. Increased lignification and cell wall thickening at GS 71 of several winter wheat cultivars, as a reaction to eyespot stem infection, have been reported (Murray & Bruehl, 1983), although it is unclear from their work which *Oculimacula* sp. was used for inoculation in the experiment.

Regressions using assessments of DI at GS 33 to predict eyespot severity at GS 60 or GS 72 for *O. yallundae* were always significant, accounting for high percentage of the variance. Similar results were obtained by Goulds & Fitt (1991a) when they compared eight different methods for measuring eyespot incidence and severity at GS 31 on winter wheat inoculated with *O. yallundae* and *O. acuformis*. They observed that, when the disease was caused by *O. yallundae*, prediction of eyespot severity at GS 71 using any of the assessments was consistently significant, accounting for more than 50% of the variance.

Regressions of disease index at GS 60 or 72 for *O. acuformis* on disease index at GS 33, although usually significant, accounted only for a small proportion of the variance. Differences in eyespot development when caused by *O. acuformis* on young and adult plants may partially explain the lack of correlation between early and late disease severity. For example , during three years of experiments on inoculated wheat with the two *Oculimacula* spp., Goulds & Fitt (1991b) observed that, in one year, *O. acuformis* grew rapidly on seedlings but failed to colonize the stems of adult plants, whilst in another year the fungus grew poorly on seedlings but produced severe lesions on the stems of adult

plants. However, the strong, significant relationship between DNA of *O. acuformis* at GS 33 and GS 60 or 72 indicates that the amounts of pathogen at later crop growth stages can be predicted. Nevertheless, the symptoms of eyespot expressed in the plants at GS 33 and 60 failed to correspond to the respective concentrations of *O. acuformis* DNA, indicating that symptom expression by this pathogen was less dependent on its DNA concentration. Asymptomatic growth of *Oculimacula* spp. at early crop growth stages has been indicated by microscopic observations (Daniels *et al.*, 1991). This may explain why, in other experiments, DNA of *O. acuformis* was detected in quantifiable amounts in the absence of visual symptoms (Nicholson & Turner, 2000). However, no reports are available on differences in the development of the two *Oculimacula* spp. in mature stems in relation to disease symptoms expression. The small percentage variances accounted for in all of the regression analyses on *O. acuformis* data, where visual assessement was used, indicate that symptoms caused by this pathogen are not reliable for predicting pathogen colonisation or disease severity late in the season. Overall, the strongest relationship for *O. acuformis* involving visual assessment was observed between eyespot score and pathogen DNA at GS 72. However, for the purpose of predicting disease or pathogen DNA late in the season, DNA quantified at GS 33 was a more reliable and consistant indicator. In comparison, visual assessment and DNA of *O. yallundae* at GS 33 proved to be similarly adequate for predicting pathogen or disease late in season.

Analyses of position and parallelism using visually assessed eyespot score data at GS 72 failed to reveal any differences between species in their effect on plant characteristics, stem bending strength or safety factor. Use of DNA quantification data, however, showed that the two *Oculimacula* spp. fitted, on most occasions parallel lines and had similar effects determined by different DNA concentrations. Crook & Ennos (1994) showed that resistance of modern winter wheat cultivars to naturally occuring stem lodging was not primarily related to the strength and stiffness of the stems but mainly to their height and weight distribution. In this study, both species reduced the lodging resistance

(stem safety factor) compared to the uninoculated control. The observed reduction in stem safety factor was associated mainly with the reduction of stem bending strength observed for the inoculated shoots (R^2=0.85, P<0.001). The main difference between the species was related to their effect on stem bending strength and analyses of position and parallelism suggested that the data were best fitted by two separate lines with different slopes and intercepts for each species. Although, regression analysis indicated that less pathogen DNA of *O. acuformis* than of *O. yallundae* was necessary to reduce stem-bending strength, the steeper line of the latter suggested that reductions caused by this pathogen will occur over smaller DNA increases.

Regressions of stem bending strength on DNA of *O. yallundae* accounted for more than 70% of the variance compared to *O. acuformis* which accounted for 50%, indicating that other factors also contributed to this effect. One such possible factor was the variation among DNA concentration of isolates of *O. acuformis* at GS 72. This is highly relevant since the differential effect of the species on stem bending strength was related to the DNA concentration of their isolates at GS 72. Poupard *et al.* (1994) also reported significant variation between individual isolates of the two *Oculimacula* spp. in their development in wheat plants at different growth stages as measured by ELISA. Differences between individual isolates of *O. acuformis* were also observed for tiller weight and self-weight moment. Isolates with greater DNA concentrations appeared to reduce tiller weight and self-weight moment of the stems more. Individual differences between isolates of *O. yallundae* in stem bending strength and safety factor appeared to be associated with the different concentrations of background DNA of *O. acuformis* detected on the same shoots. Reductions in stem bending strength and safety factor were greatest on shoots inoculated with individual *O. yallundae* isolates where less background DNA of *O. acuformis* was detected. Since no difference between isolates of *O. yallundae* for DNA concentration at GS 72 was found, the exact reason for the observed differences is unclear but it is possible that some isolates were less aggressive and more tolerant of secondary infections by

isolates of *O. acuformis*. Thus, the overall effects of each *Oculimacula* sp. on those plant characteristics and factors associated with lodging, where significant isolate variation was found, was determined by the intinsic aggressiveness of individual isolates within the species. To clarify these effects, a larger number of isolates would be required in further work.

The observed reduction in tiller number in inoculated plots at GS 33 supports early reports on this effect of severe eyespot on winter wheat (Sprague, 1934; Bruehl *et al.*, 1968; Scott & Hollins, 1974). It is likely that the effects of inoculation with *O. acuformis* and *O. yallundae* on ear weight, tiller weight and height would have been larger if the comparison was made with disease-free plants. In the present study, however, inoculum moved freely between plots early in the season. Therefore, although initially small pathogen DNA concentrations were detected in the control, the DNA amount of both species exceeded 40 pg ng^{-1} of total DNA by GS 72.

Slight and moderate lesions caused by the species had no effect on ear weight as indicated by the analysis of averaged values per plot. However, severe lesions caused by *O. acuformis* and *O. yallundae* reduced ear weight by 3 and 7%, respectively, corresponding to more than 60% more pathogen DNA concentration than in the control. This is in agreement with previous reports that losses mesured on single stems are mainly due to severe lesions and not to slight or moderate lesions caused by *Oculimacula* spp. (Jørgensen, 1964; Scott & Hollins, 1974).

Previous studies (Glynne *et al.*, 1945; Scott & Hollins, 1974) have demonstrated that results derived from single-stem observations may not represent the actual effect of the disease on the whole crop or plants where compensation may occur from the less diseased plants and tillers. In this study, such compensation effects were not observed for *O. acuformis*, which reduced yield by 11%. Although plots inoculated with *O. yallundae* had about 6% less yield than the control, the difference was not statistically significant. The large differences between species in their DNA concentrations at GS 60 indicated that the

disease developed more rapidly in the *O. acuformis*-inoculated plots. Therefore, it is possible that infection by *O. acuformis* had spread onto more tillers than *O. yallundae*, where yield loss may have been partially compensated by the less-infected neighbouring tillers. The yield losses of 11% for *O. acuformis* and even 6% for *O. yallundae* are in agreement with losses of 11-12% and between 7-10% for eyespot in inoculated experiments in the absence of lodging reported by Scott & Hollins (1974) and Jørgensen (1964), respectively.

Other studies (Scott & Hollins, 1974; Clarkson, 1981) have demonstrated from single-stem measurements, that eyespot reduced thousand grain weight and grain number per ear in winter wheat. However, in this study, such effects were not apparent on an individual plot basis and eyespot caused by either species had no effect on thousand-grain weight or specific weight.

The main findings of this work are that *O. acuformis* can cause severe yield loss of winter wheat and that effects on plant characteristics associated with lodging of both species are similar but dependent on different pathogen DNA concentrations. The effects of the disease when caused by *O. acuformis* as demonstrated in this study require to be confirmed by further work with inoculated plots in experiments using different cultivars.

Both species demonstrated an ability to reduce the stem safety factor by reducing stem bending strength. In comparison, the safety factor of uninfected crops to naturally occuring lodging would be largely dependent on the self-weight moment of plants (Crook & Ennos, 1994). Severe eyespot caused by both species can increase the risk of lodging by reducing stem strength significantly.

The effects on lodging and plant characteristics associated with it may also be influenced by the aggressiveness of individual isolates within the species. Thus, some of the isolates of *O. acuformis* and *O. yallundae* may be more damaging than others. The significant isolate variation found within *O. acuformis* and *O. yallundae* at GS 72

suggests that, for inoculated experiments the selection of isolates for eyespot inoculum is important in order to obtain representative results.

Prediction of disease or pathogen DNA late in the season using visual assesment was consistent only for disease caused by *O. yallundae*. Prediction of the amount of *O. acuformis* late in the season was more reliable using pathogen DNA at GS 33 rather than visiual assessment at the same growth stage. Although yield loss correlated significantly with DNA of *O. acuformis* at GS 33, only a small percentage of the variance was accounted for by the regression, indicating that other factors were important and pathogen DNA at GS 33 is not a reliable indicator of yield loss. Prediction of yield loss was improved by using DNA of *O. acuformis* at GS 72 where the regression accounted for 38% of the variance.

Based on the findings in this study the original null hypothesis regarding the effect of eyespot on lodging resistance of wheat was rejected, whilst the null hypothesis for differences between species on their effect on lodging resistance and yield loss in winter wheat had to be accepted.

CHAPTER 4

Effects of fungicides on eyespot, caused predominantly by *Oculimacula acuformis*, and yield of early-drilled winter wheat

INTRODUCTION

Second and third successive cereal crops are often considered to be at a greater risk from eyespot than those following a non-cereal break crop, although disease epidemics are also influenced by other factors such as environment, sowing date or tillage practices (Colbach *et al.*, 1997).

Decisions on fungicide use for eyespot control in the UK are based on an economic threshold of more than 20% of infected shoots showing penetrating lesions (Anon, 1986) at growth stages (GS) 30-31 (Zadoks *et al.*, 1974). This threshold was established when *O. yallundae* was the predominant pathogen responsible for the disease. However, since the 1980s, the eyespot fungus population has changed and at present the dominant pathogen causing the disease in the UK is *O. acuformis* (King & Griffin, 1985; West *et al.*, 1998, Nicholson & Turner, 2000). The shift in pathogen population may have significant implications for the validity of the 20% threshold since symptoms caused by *O. acuformis* often develop more slowly and are expressed later than those caused by *O. yallundae* (Goulds & Fitt, 1991b). As a result, few if any visible symptoms of eyespot caused by *O. acuformis* can be observed as early as GS 30, thus confounding decisions on the need for fungicide control. The accuracy of visual disease assessment for eyespot at GS 30-31 may be further weakened as the disease frequently occurs with brown foot rot and/or sharp eyespot, and mixed infections commonly obscure the early symptoms of eyespot (Turner *et al.*, 2001). Currently, PCR methods are often used in research to diagnose positively and to quantify the main pathogens present on the stems, including *Microdochium nivale* and *Fusarium* spp., causing brown foot rot, and *Rhizoctonia cerealis*, causing sharp eyespot.

Responses to eyespot fungicides are variable, partly reflecting differences in amounts of the disease present in the crop (Morgan *et al.*, 1998) or differences in the susceptibilities of different cultivars (Nicholson & Turner, 2000). Which of the two species is the dominant pathogen causing the disease can also influence the performance of

some fungicides. For example, Bateman *et al.* (2000a) reported that prochloraz was more effective on sites where *O. yallundae* predominated, and that relatively good control of eyespot was sometimes associated with significant amounts of rainfall following its application. Both of the *Oculimacula* spp. are capable of causing severe disease at the end of the season and recent work has indicated that symptoms of similar severity caused by the two pathogens, if attained, are likely to be equally damaging (Bateman & Jenkyn, 2001). It has, however, been suggested that there may be less need for fungicide treatment where eyespot is caused by *O. acuformis* because the slower penetration and later development of this species mean that it is less likely to become damaging (Royle, 1998). It is, therefore, important to clarify the effects of fungicides on eyespot disease in winter wheat caused predominantly by *O. acuformis*, which is the main causal agent of the disease in the UK at present.

The objectives of the study were to evaluate the effectiveness of nine fungicide treatments against eyespot, caused predominantly by *O. acuformis*, using visual and PCR methods, and also to investigate relationships between eyespot incidence or severity, fungal biomass estimated by quantitative PCR, and yield. Effects of the fungicides on brown foot rot and sharp eyespot, and their causal pathogens (*Fusarium* spp., *Microdochium nivale* and *Rhizoctonia cerealis*, respectively) were also measured using quantitative PCR.

Null hypothesis tested: Fungicides applied at GS 31 have no effect on eyespot caused by *O. acuformis* and yield of early-drilled winter wheat.

MATERIALS AND METHODS

Field experiments

Similar experiments were carried out in two years on three different sites of commercially grown winter wheat in Shropshire. Each experiment had four randomised blocks of 10 plots (each 12 m x 3 m) in which the effects of 10 fungicide treatments (including an untreated control) were compared. A different cultivar of winter wheat was used at each site. Consort was grown as an early-drilled first winter wheat, following potatoes, preceded by winter wheat. Savannah was drilled as a second winter wheat, preceded by potatoes and Claire as a third cereal, following oats, preceded by winter wheat. By using cultivars with different resistances to eyespot (Claire having the highest resistance rating of 7 and Consort and Savannah having resistance ratings of 6; Anon., 2002), drilled at different stages in a potatoes/cereals rotation, it was anticipated that a range of eyespot disease severities would be established on which to test the effects of the fungicides (Table 4.1). Winter wheat cultivars with a wider range of resistance ratings to the disease are not currently available commercially.

Savannah, Consort and Claire were sown on 1 September 2000, 8 September 2000 and 14 September 2001, respectively. Fungicide treatments and plant growth regulator (chlormequat at a field rate of 1.25 l ha^{-1} as Cycocel® and trinexapac-ethyl at a field rate of 0.2 l ha^{-1}as Moddus®) were applied at GS 31 after the first crop sampling as described in Chapter 2. Overall fungicide applications were made at GS 39 of each cultivar to control foliar diseases. Consort and Claire received epoxiconazole, fenpropimorph and kresoxim-methyl as Mantra® at a field rate of 0.7 l $ha^{-1}$and 0.5 l ha^{-1}, respectively. Savannah received an application of tebuconazole and azoxystrobin as Folicur® and Amistar®, respectively, each at a field rate of 0.5 l ha^{-1}. The only foliar diseases present on the cultivars were septoria leaf spot and mildew, which were successfully controlled at GS 39

and no further fungicide applications were needed. All other crop husbandry is listed in Appendix 10.

Plots were harvested using a small-plot combine harvester (Winterstieger Crop Master), and grain yields (at 15% moisture content), thousand grain weights and specific (hectolitre) weights were measured.

Sampling and disease assessments

Thirty plants were collected at random from each plot at GS 31, 39 and 69, except for Claire for which the second sampling was at GS 55 and the third at GS 70. In order to allow time for pathogen DNA recovery and accumulation following the application of fungicide treatments, the crops were sampled at approximately 5 to 6 week intervals. Claire advanced much faster up to GS 39 due to a warmer season, and in order to allow the same interval between samples as for the other two cultivars it was sampled at GS 55. For samples taken at GS 31 and 39/55, visual assessments of eyespot and other stem-base diseases were made on the main shoots and all tillers. For samples collected at GS 69/70, only symptoms on the main shoot were assessed. Disease assessments were carried out as described in Chapter 2. The incidence of symptoms identified as eyespot, sharp eyespot and brown foot rot was recorded for each plant at GS 31 and 39/55 and for each main shoot of each plant at GS 69/70.

Table 4.1. Fungicide treatments and rates of application at GS 31.

Treatment	Active ingredient (a.i.)	Trade name	Rate g a.i. ha^{-1}
1	-	Untreated	-
2	Epoxiconazole	Opus	62.5
3	Kresoxim methyl + Epoxiconazole	Landmark	62.5 + 62.5
4	Prochloraz	Sportak	405
5	Prochloraz + Epoxiconazole	Sportak + Opus	405 + 62.5
6	Prochloraz + Fluquinconazole	Foil	156.6 + 48.6
7	Cyprodinil	Unix	750
8	Cyprodinil + Epoxiconazole	Unix + Opus	750 + 62.5
9	Cyprodinil + Epoxiconazole	Unix + Opus	500 + 62.5
10	Cyprodinil + Epoxiconazole + Picoxystrobin	Unix + Opus + Acanto	500 + 62.5 + 150

Stem bases used for disease assessments were prepared immediately afterwards for DNA extraction. The basal region (4 cm length) of each stem was chopped finely, freeze-dried and milled to a powder in a ball mill (Griffin, UK). DNA was extracted and quantified as described in Chapter 2.

Oligonucleotide primer sequences specific to *O. acuformis*, *O. yallundae* (Beck *et al.*, 1996), *R. cerealis* (US patent No. 6,485,907), *M. nivale* (*M. nivale* var. *majus* and var. *nivale*, JB612 forward primer & ITS-4 reverse primer) and *Fusarium* spp. (JB566 forward primer & JB572 reverse primer) (US Patent No. 5,815,453, Beck, 1997) were obtained from Syngenta Biotechnology Inc. (J. Beck pers. comm., November 2000) (Chapter 2). Competitor fragments for quantitative PCR for *O. acuformis*, *O. yallundae* and *R. cerealis* were constructed as described in Chapter 2. Competitor fragments for quantitative PCR for *M. nivale*, *Fusarium* spp. were supplied by S. G. Edwards, Harper Adams University College, UK. Total DNA stocks of plant samples at 4 or 40 ng μl^{-1} were amplified using a PTC-100 thermal cycler. Amplification conditions and programmes used for *O. acuformis*, *O. yallundae* and *R. cerealis*, *M. nivale* and *Fusarium* spp are listed in Chapter 2. Fungal DNA standards were prepared as described in Chapter 2. Following gel electrophoresis, gels were viewed under UV light on a Gel Doc 1000 fluorescent gel documentation system (Bio-Rad Laboratories Ltd, Hemel Hempstead, UK), and unsaturated images were analysed using Molecular Analyst software (Bio-Rad). PCR product ratios were determined for each standard and sample by dividing the band intensity of pathogen DNA product by that of the internal standard product. Standard curves were generated using the genomic DNA of each pathogen and the unit of DNA quantified was picogrammes (pg) of pathogen DNA per nanogramme (ng) of total DNA (plant and fungal).

Statistical analyses

Data were analysed using Genstat (version 5 release 4.1, Lawes Agricultural Trust, IACR Rothamsted, UK). Where necessary, log_{10} transformation was used for DNA data in order to obtain normal distribution. Relationships between disease index and pathogen DNA were determined by single linear regression analyses, with effects considered significant where $P \leq 0.05$.

RESULTS

Effects of fungicides on incidence and disease index of stem-base diseases

Prior to fungicide applications at GS 31, visual assessments of stem-base diseases revealed no significant differences between plots due to receive different treatments, with average eyespot disease index (DI) of 23%, 28% and 22% for Consort, Savannah and Claire, respectively. In Consort, there were significant reductions in eyespot DI at GS 39, where mixtures of cyprodinil and epoxiconazole were applied compared to the untreated control (Table 4.2). In the same cultivar at GS 69, all treatments containing cyprodinil significantly reduced eyespot DI, but it was, again, more effective when mixed with epoxiconazole, reducing eyespot DI by more than 50%. In the cultivar Savannah, cyprodinil mixed with epoxiconazole alone or with epoxiconazole and picoxystrobin, significantly reduced eyespot DI at GS 69 by 52% and 39%, respectively. Prochloraz or epoxiconazole alone failed to reduce eyespot DI at either GS 39 or 69 in either Consort or Savannah. In the cultivar Claire, there were no significant effects on the incidence or DI of any of the stem base diseases at any growth stage.

Visually assessed brown foot rot and sharp eyespot had higher disease indices in Consort than in Savannah or Claire. The mean disease indices at GS 69/70 of Consort, Savannah and Claire were 9.3, 2.0 and 1.3 for brown foot rot and, 3.9, 1.4 and 4.7 for sharp eyespot, respectively. There were no significant effects of treatments on the incidence or severity of brown foot rot and sharp eyespot in Savannah or Claire at any growth stages or in Consort at GS 31 or 69 (Table 4.3). However, in Consort at GS 39, all treatments that included cyprodinil significantly reduced brown foot rot DI by an average of 45% (results not shown). This effect was not confirmed by quantitative PCR of *M. nivale*.

Effect of fungicides on pathogen DNA

PCR assays showed that *O. acuformis* was the dominant eyespot pathogen at GS 31 in all three cultivars (Table 4.4). Significant effects of treatments were observed on DNA of *O. acuformis*_at GS 39 and 55 for Consort and Claire, respectively, and at GS69/70 for all three cultivars (Table 4.5). In Consort at GS 39, only treatments containing cyprodinil provided significant reductions in DNA of *O. acuformis*. However, in Claire at GS 55, all treatments except the mixture of kresoxim-methyl and epoxiconazole significantly reduced DNA of *O. acuformis* compared to the untreated control.

At GS 69 of Consort, all treatments containing cyprodinil resulted in less DNA of *O. acuformis* in stems than any other treatment. Similarly, in Savannah at GS 69, all treatments that included cyprodinil, except cyprodinil, epoxiconazole and picoxystrobin reduced significantly the DNA of *O. acuformis* compared to the untreated control. However, in Claire at GS 70, treatment effects were not as consistent as in Consort and Savannah. Prochloraz, alone or in combination with epoxiconazole, and cyprodinil, applied at the lower rate in combination with epoxiconazole or epoxiconazole and picoxystrobin were the only treatments significantly reducing the levels of *O. acuformis*, with the latter providing 70% reduction of DNA compared with the untreated control.

Oculimacula yallundae was not detected in the cultivar Savannah. It was found in the crops of Consort and Claire (Table 4.4), but its distribution was patchy and there were no significant differences between treatments.

There were no significant effects of treatments on amounts of DNA of the pathogens that cause brown foot rot or sharp eyespot at any growth stage in any cultivars (Table 4.4). In all three cultivars, *M. nivale* was the main pathogen associated with brown foot rot. More DNA of *M. nivale* was found in Claire than in the other two cultivars at all sampling times. DNA of *R. cerealis* was found in relatively higher concentrations than DNA of *M. nivale* in both Consort and Savannah at GS 39 and 69.

Effect of fungicides on yield and thousand grain weight

All treatments significantly increased yield of Consort and the combination of cyprodinil, epoxiconazole and picoxystrobin provided the greatest yield increase of 17% above the untreated control (Table 4.6). There were similar effects on yield of Claire, except that applying prochloraz or cyprodinil on their own gave yield increases that were only small and not significant

There were no significant effects on TGW of Consort, but all treatments except prochloraz plus fluquinconazole significantly increased TGW of Claire (Table 4.6). Fungicides had no significant effects on specific weight of Consort or Claire (means of 73.00 kg hl^{-1} and 76.95 kg hl^{-1}, respectively).

Yield, specific weight and TGW were not determined for Savannah because the experiment was harvested erroneously by the farmer's contractor.

Table 4.2. Effects of fungicides applied at GS 31 on eyespot disease index.

Treatment[1]	Eyespot disease index					
	GS 39 Consort	GS 39 Savannah	GS 55 Claire	GS 69 Consort	GS 69 Savannah	GS 70 Claire
1	21.7	12.2	55.6	55.0	37.8	62.8
2	20.8	15.0	52.8	50.6	29.7	59.2
3	19.7	15.6	52.5	54.4	32.2	53.6
4	21.1	20.8	43.0	55.3	32.8	54.4
5	23.6	11.9	39.2	50.6	35.0	46.4
6	19.4	19.2	44.2	53.1	34.7	46.1
7	12.5	11.1	35.3	35.6	30.6	45.0
8	8.6	9.7	31.1	19.2	18.3	53.1
9	7.2	11.9	36.1	21.9	18.3	51.9
10	5.8	16.9	33.1	21.4	23.1	30.8
SED	5.86	5.61	8.78	8.98	5.92	9.54
LSD	12.02	11.50	18.01	18.40	12.14	19.58
P	0.023	0.570	0.076	0.001	0.020	0.128

[1] 1-untreated, 2-epoxiconazole (62.5 g a.i. ha^{-1}), 3-kresoxim methyl (62.5 g a.i. ha^{-1}) + epoxiconazole (62.5g a.i. ha^{-1}), 4-prochloraz (405 g a.i. ha^{-1}), 5-prochloraz (405 g a.i. ha^{-1}) + epoxiconazole (62.5 g a.i. ha^{-1}), 6-prochloraz (156.6 g a.i. ha^{-1}) + fluquinconazole (48.6 g a.i. ha^{-1}), 7-cyprodinil (750 g a.i. ha^{-1}), 8- cyprodinil (750 g a.i. ha^{-1}) + epoxiconazole (62.5 g a.i. ha^{-1}), 9- cyprodinil (500 g a.i. ha^{-1}) + epoxiconazole (62.5 g a.i. ha^{-1}), 10- cyprodinil (500 g a.i. ha^{-1}) + epoxiconazole (62.5 g a.i. ha^{-1}) + picoxystrobin (150 g a.i. ha^{-1})

Table 4.3. Mean brown foot rot and sharp eyespot disease index.

		Disease index					
		GS 31		GS 39 /55		GS 69 /70	
Year	Cultivar	BFR[1]	SE[2]	BFR	SE	BFR	SE
2000	Consort	19.5	22.0	16.0	7.4	9.3	6.9
s.e.d.		6.1	5.7	4.7	4.9	4.9	4.2
2000	Savannah	14.3	2.9	3.5	0.7	2.0	1.4
s.e.d.		4.7	1.5	2.3	0.7	1.4	1.7
2001	Claire	6.1	0.8	6.8	7.6	1.3	4.7
s.e.d.		1.9	0.8	2.2	5.1	1.1	3.2

[1] Brown foot rot, [2] Sharp eyespot

Table 4.4. Mean amounts of pathogen DNA in stem bases at each growth stage.

Year	Cultivar	DNA (pg ng $^{-1}$ of total DNA)														
		GS 31					GS 39 / 55					GS 69 / 70				
		O. a[1]	*O. y*[2]	*R. c*[3]	*M. n*[4]	*F*[5]	*O. a*	*O. y*	*R. c*	*M. n*	*F*	*O. a*	*O. y*	*R. c*	*M. n*	*F*
2000	Consort	0.12	-	-	-	-	3.45	0.52	6.41	0.27	-	33.50	9.07	9.00	0.59	-
s.e.d.		0.05					1.24	0.21	3.28	0.15		16.10	12.95	8.91	0.58	
2000	Savannah	0.33	-	0.73	0.18	-	5.06	-	26.8	2.35	-	10.40	-	6.33	1.74	-
s.e.d.		0.32		0.54	0.22		2.76		13.26	1.51		7.29		2.97	2.1	
2001	Claire	1.55	1.11	1.44	0.48	0.03	1.44	0.69	4.25	7.24	0.33	5.94	7.40	9.80	10.13	0.26
s.e.d.		0.79	3.83	1.44	0.45	0.03	0.47	0.58	2.90	4.31	0.41	1.99	7.08	5.42	4.32	0.20

[1] *Oculimacula acuformis*, [2] *Oculimacula yallundae*, [3] *Rhizoctonia cerealis*, [4] *Microdochium nivale*, [5] *Fusarium* spp.

-, not detectable

Table 4.5. Effects of fungicides applied at GS 31 on DNA of *O. acuformis*.

	log $_{10}$ DNA of *O. acuformis* (pg ng^{-1} of total DNA, back-transformed means are shown in parentheses)					
Treatment[1]	GS 39 Consort	GS 39 Savannah	GS 55[2] Claire	GS 69 Consort	GS 69 Savannah	GS 70 Claire
1	0.67 (4.72)	0.46 (2.90)	2.46	1.70 (50.00)	0.93 (8.51)	0.99 (9.77)
2	0.46 (2.91)	0.56 (3.61)	2.28	1.68 (47.42)	1.21 (16.21)	0.77 (5.82)
3	0.55 (3.52)	0.72 (5.27)	1.37	1.62 (41.78)	1.09 (12.30)	0.77 (5.92)
4	0.61 (4.03)	0.69 (4.84)	1.27	1.78 (59.57)	1.08 (12.02)	0.61 (4.05)
5	0.51 (3.24)	0.46 (2.88)	1.38	1.65 (44.67)	0.92 (8.31)	0.59 (3.91)
6	0.60 (4.00)	0.57 (3.67)	1.40	1.59 (38.90)	1.29 (19.50)	0.88 (7.59)
7	0.43 (2.66)	0.25 (1.78)	0.73	0.63 (4.29)	-0.49 (0.32)	0.77 (5.92)
8	0.27 (1.85)	0.46 (2.88)	1.34	0.16 (1.46)	-1.26 (0.05)	0.74 (5.53)
9	0.47 (2.92)	0.51 (3.24)	1.31	0.76 (5.79)	-0.87 (0.13)	0.68 (4.83)
10	0.44 (2.75)	0.72 (5.20)	0.83	0.43 (2.68)	0.34 (2.18)	0.24 (1.72)
SED	0.11	0.26	0.47	0.24	0.47	0.15
LSD	0.22	0.53	0.97	0.49	0.96	0.31
P	0.039	0.750	0.024	< 0.001	< 0.001	0.005

[1] 1-untreated, 2-epoxiconazole (62.5 g a.i. ha^{-1}), 3-kresoxim methyl (62.5 g a.i. ha^{-1}) + epoxiconazole (62.5g a.i. ha^{-1}), 4-prochloraz (405 g a.i. ha^{-1}), 5-prochloraz (405 g a.i. ha^{-1}) + epoxiconazole (62.5 g a.i. ha^{-1}), 6-prochloraz (156.6 g a.i. ha^{-1}) + fluquinconazole (48.6 g a.i. ha^{-1}), 7-cyprodinil (750 g a.i. ha^{-1}), 8- cyprodinil (750 g a.i. ha^{-1}) + epoxiconazole (62.5 g a.i. ha^{-1}), 9- cyprodinil (500 g a.i. ha^{-1}) + epoxiconazole (62.5 g a.i. ha^{-1}), 10- cyprodinil (500 g a.i. ha^{-1}) + epoxiconazole (62.5 g a.i. ha^{-1}) + picoxystrobin (150 g a.i. ha^{-1})

[2] normally distributed untransformed data

Table 4.6. Yield and thousand grain weight (TGW) for each treatment, applied to cultivars.

	Yield (t ha $^{-1}$)		TGW (g)	
Treatment[1]	Consort	Claire	Consort	Claire
1	10.05	10.25	47.15	45.47
2	11.02	10.72	48.71	47.91
3	11.16	10.72	49.47	47.94
4	10.49	10.57	47.64	48.49
5	11.19	11.02	48.83	49.26
6	10.67	10.74	48.64	47.09
7	10.75	10.54	47.56	47.34
8	11.38	10.77	49.80	49.22
9	11.43	10.97	49.49	48.47
10	11.76	11.12	49.98	48.34
SED	0.15	0.22	1.00	0.86
LSD	0.32	0.46	2.06	1.76
P	< 0.001	0.027	0.085	0.006

[1] 1-untreated, 2-epoxiconazole (62.5 g a.i. ha^{-1}), 3-kresoxim methyl (62.5 g a.i. ha^{-1}) + epoxiconazole (62.5g a.i. ha^{-1}), 4-prochloraz (405 g a.i. ha^{-1}), 5-prochloraz (405 g a.i. ha^{-1}) + epoxiconazole (62.5 g a.i. ha^{-1}), 6-prochloraz (156.6 g a.i. ha^{-1}) + fluquinconazole (48.6 g a.i. ha^{-1}), 7-cyprodinil (750 g a.i. ha^{-1}), 8- cyprodinil (750 g a.i. ha^{-1}) + epoxiconazole (62.5 g a.i. ha^{-1}), 9- cyprodinil (500 g a.i. ha^{-1}) + epoxiconazole (62.5 g a.i. ha^{-1}), 10- cyprodinil (500 g a.i. ha^{-1}) + epoxiconazole (62.5 g a.i. ha^{-1}) + picoxystrobin (150 g a.i. ha^{-1})

Regression analyses, using individual plot data, revealed usually significant but weak relationships between the amounts of *O. acuformis* DNA in the stem bases and eyespot incidence at the same growth stage (Table 4.7). The significances of these regressions were generally greater at the later growth stages when visual symptoms were more easily distinguished. Significant positive relationships were found between eyespot incidence and DNA of *O. acuformis* at GS 69/70 for all cultivars (Table 4.8). This relationship was weaker for Savannah and Claire where symptoms of eyespot may have been obscured by brown foot rot and/or sharp eyespot. DNA of *O. acuformis* at GS 39/55 correlated positively with DNA of *O. acuformis* at GS 69/70 for all cultivars but the regression was not significant for Savannah, indicating that pathogen DNA concentration at GS 69 was influenced by other factors (Table 4.8).

Regression analysis of the mean grain yield for each treatment on eyespot incidence was significant only for Consort, but regressions of yield on DNA of *O. acuformis* at GS 69/70 revealed negative relationships, which were significant for both Consort and Claire (Table 4.8).

Table 4.7. Summary of regressions of eyespot incidence on the amounts of DNA of *O. acuformis* at each of three growth stages for cultivars Consort, Savannah and Claire.

Cultivar	Year	Growth stage	Regression equation	Variance accounted for %	P
Consort	2000	31	y = 32.34 + 104.3x	26.6	< 0.001
Savannah	2000	31	y = 44.49 + 11.53x	10.2	0.03
Claire	2001	31	y = 30.57 + 0.49x	-	0.71
Consort	2000	39	y = 20.46 + 0.37x	-	0.70
Savannah	2000	39	y = 18.41 - 0.476x	3.0	0.15
Claire	2001	55	y = 40.85 + 9.14x	18.6	0.003
Consort	2000	69	y = 34.67 + 0.40x	36.1	< 0.001
Savannah	2000	69	y = 27.30 + 0.35x	13.0	0.01
Claire	2001	70	y = 49.62 + 2.00x	15.6	0.01

Table 4.8. Summary of regressions for cultivars Consort, Savannah and Claire.

Cultivar	Year	Response variate	Independent variate	Regression equation	Variance accounted for %	P
Consort	2000	EI at GS69	*O. a*[2] DNA at GS 69	y = 26.14 + 0.66x	89	< 0.001
Savannah	2000	EI at GS 69	*O. a* DNA at GS 69	y = 25.10 + 0.56x	36	0.04
Claire	2001	EI at GS 69	*O. a* DNA at GS 70	y = 43.49 + 3.03x	43	0.02
Consort	2000	*O. a* DNA at GS 69	*O. a* DNA at GS 39	y = -42.30 + 21.95x	66	0.002
Savannah	2000	*O. a* DNA at GS 69	*O. a* DNA at GS 39	y = -13.50 + 4.72x	16	0.14
Claire	2001	*O. a* DNA at GS 70	*O. a* DNA at GS 55	y = 2.28 + 2.54x	32	0.05
Consort	2000	Yield	EI[1] at GS 69	y = 11.97 - 0.02x	43	0.02
Claire	2001	Yield	EI at GS 69	y = 11.75 - 0.02x	25	0.08
Consort	2000	Yield	*O. a* DNA at GS 69	y = 11.49 - 0.01x	49	0.02
Claire	2001	Yield	*O. a* DNA at GS 70	y = 11.42 - 0.11x	79	< 0.001

[1] eyespot incidence

[2] *O. acuformis*

DISCUSSION

The null hypothesis set was that fungicides applied at GS 31 have no effect on eyespot caused by *O. acuformis* and yield of early-drilled winter wheat. However, fungicide treatments containing cyprodinil successfully reduced DNA of *O. acuformis*, resulting in decreased eyespot DI. Therefore the null hypothesis was rejected. Bateman *et al.* (2000a) and Burnett *et al.* (2000), have also reported effective control of eyespot by cyprodinil. Prochloraz alone or in mixtures failed to reduce DNA of *O. acuformis* or any other pathogen, except in the case of Claire where significant decreases were achieved when it was applied alone or with epoxiconazole. This inconsistency of the performance of prochloraz against eyespot has been partially explained previously by its reliance on effective redistribution after chemical application from foliage to stem base by rainfall (Cooke *et al.*, 1989). Daniels & Lucas (1990) also found that prochloraz was more effective when applied as a protective spray prior to inoculation than as curative spray, because of re-growth of the pathogen from structures within the host tissues, not directly exposed to the fungicide. Furthermore, Burnett *et al.* (1997) demonstrated that prochloraz was more effective against *O. acuformis* applied just before GS 30 compared to cyprodinil which had an optimum timing at GS 32. Although no resistance of *O. acuformis* to prochloraz has been reported in the UK, Bateman (2002) found a greater range of sensitivities to prochloraz among isolates of *O. acuformis* than of *O. yallundae*, which resulted in gradual selection of *O. acuformis*. Intrinsically less-sensitive isolates of *O. acuformis* to triazoles have been detected in France (Leroux, 1998) and, in our study, epoxiconazole alone, or fluquinconazole in a mixture, were not effective in reducing DNA of *O. acuformis* on any cultivar.

Fungicide treatments showed no significant effect on brown foot rot or sharp eyespot incidence, DI or pathogen DNA. Previously, other researchers observed that

successful fungicidal control of one of the stem base diseases often resulted in the increase of another. Reinecke *et al.* (1979) reported a negative relationship between eyespot and sharp eyespot, where fungicides that effectively controlled eyespot caused increases in sharp eyespot. However, no such relationship was observed in this study.

Eyespot caused by *O. acuformis* was the only disease found on Consort at GS 31, confirmed by quantitative PCR, which was grown after a one-year cereal break. Ploughing the soil before sowing may have brought infected crop residues up to the soil surface thus increasing the available eyespot inoculum. This is in agreement with previous reports by Colbach & Maynard (1995) that, where a host crop was grown with one-year break, soil inversion increased the primary infection risk. *Oculimacula acuformis* was present in the stem bases of all three cultivars in sufficient amounts to cause severe eyespot, indicating that place in the rotation had little effect on the infection and development of the disease in this limited comparison. Similarly, Bardsley *et al.* (1998) reported that previous cropping had little influence on the incidence and severity of eyespot, caused by *O. acuformis.* *Oculimacula yallundae*, *R. cerealis* and *M. nivale* were not detected on Consort before GS 39. The absence of other stem-base pathogens early in the season is likely to have encouraged the development of *O. acuformis* and established the dominance of this pathogen. Alternatively, on the second and third wheat crops, Savannah and Claire respectively, foot rot and sharp eyespot were found as early as eyespot. *Microdochium nivale* and *R. cerealis* were detected as early as GS 31 and, by GS 39, DNA amounts were similar to or higher than amounts of *O. acuformis*. Thus, in the second and third wheats in which all the pathogens of the stem-base disease complex were present as early as GS 31, microbial interactions such as competition may have resulted in slower development of *O. acuformis* on the stem bases. There is no apparent reason for the absence of *O. yallundae* on the wheat stem bases at GS 31 of Consort and Savannah or for its relatively smaller DNA amounts in Claire. It is possible that fungicides such as prochloraz were used in previous years, selecting for *O. acuformis* (Bateman, 2002), which then occurred in

relatively larger proportions and was more evenly spread across the fields. Under these conditions, the fungicides failed to show any significant reduction in DNA of *O. yallundae*.

Yield increases due to the effective control of eyespot by cyprodinil were clearly seen on Consort, where disease pressure was greatest, and *O. acuformis* was found in larger amounts, especially at the end of the growth season. Although prochloraz and cyprodinil, applied alone, failed to improve the overall grain yield of Claire, the same fungicides raised TGW significantly above that of the untreated control. The mixture of cyprodinil, epoxiconazole and picoxystrobin increased yield by a greater amount than any other treatments in Consort, or cyprodinil alone and prochloraz alone in Claire. The increases in yield of Consort and Claire where treatments failed to control eyespot were associated with their activity against early foliar diseases such as septoria leaf spot, and the addition of cyprodinil to epoxiconazole, for example, provided up to 4% additional yield increase.

Regression analysis of yield on DNA of *O. acuformis* indicated that both cultivars will suffer potential yield losses depending on the amounts of pathogen DNA. The variable effect of fungicides against eyespot caused by *O. acuformis* on Claire may have resulted because of the relatively smaller amounts of pathogen present on the plants. Furthermore, as indicated by regression analysis of *O. acuformis* DNA at GS 69/70 on DNA at 39/55 it appeared that the rate of accumulation of pathogen DNA on stem bases of Savannah and Claire was less than in Consort. Greater resistance to eyespot of Claire, in agreement with NIAB ratings, would explain the slower accumulation of DNA of *O. acuformis*. However, it is more likely that the development of *O. acuformis* was slower because of suppression by other stem-base pathogens, found in larger amounts on Savannah and Claire, compared to Consort.

The major pathogen causing brown foot rot was *M. nivale* on all cultivars. In one instance, Consort at GS 39, there was significant reduction of brown foot rot DI by cyprodinil. However, this was not confirmed by quantitative PCR, indicating possible

misidentification with eyespot symptoms. Early symptoms of both eyespot and brown foot rot can be similar and often *M. nivale* and *Oculimacula* spp. occur close together on stems making accurate identification difficult. Bateman (1993) isolated significant amounts of both *Oculimacula* spp. from brown foot rot lesions. Turner *et al.* (1999) also found an association between *Oculimacula* spp. and brown foot rot symptoms, in particular between *M. nivale* var. *majus* and *O. acuformis*, indicating that these two species may be capable of coexisting in lesions, including those of indeterminate type. Bateman *et al.* (2000a & b) and Turner *et al.* (2002) found that the co-occurrence of the two pathogens on the same stem bases tended to be more frequent than would have occurred by chance. However, in these studies, there was no clear indication of any interactions between the stem–base pathogens.

Correlations between visually assessed disease incidence or severity and DNA amounts at GS 31 and 69 were weak, indicating that prediction of disease development based on visual assessment would be inaccurate. Improved relationships for DNA accumulation after GS 39/55 were observed indicating that possibly GS 39 is the earliest time pathogen DNA can be used as a predictor of DNA concentrations at the end of the growing season. The absence of a significant relationship between pathogen DNA at GS 31 and 39 suggests that disease development for this period is less likely to be dependent on the initial disease amounts present at the GS 31 and more likely to be influenced by other factors. Indeed, there is much evidence available at present indicating that these relationships are largely influenced by year and location, probably due to climatic and/or agronomic differences (Turner *et al.*, 2001).

In conclusion, this study indicates that effective eyespot control resulting in significant yield increases could be achieved by using fungicide mixtures inclusive of cyprodinil, particularly in situations where *O. acuformis* is dominating the pathogen populations on the stem bases early in season. Under such circumstances, *O. acuformis* was capable of causing yield or TGW losses on the cultivars used in this study. The

potential of *O. acuformis* to cause yield loss was supported by the significant negative correlation between yield and disease incidence or pathogen DNA quantified at GS 69. Both visual and molecular methods failed to predict eyespot disease incidence or pathogen DNA late in season using incidence or DNA amounts at GS 31. However, PCR assays used at GS 31 accurately identified stem-base pathogens where early symptoms of individual diseases were difficult to distinguish.

CHAPTER 5

Studies on the relationships between *Microdochium nivale*, *Oculimacula acuformis* and *O. yallundae* and the development of eyespot and brown foot rot in winter wheat

INTRODUCTION

Eyespot and brown foot rot frequently occur simultaneously on the stem bases of cereals causing indistinct symptoms and thus impeding the visual assessment of eyespot when spray decisions are made at GS 30/32 (Anon., 1986). Although no direct evidence is available for the existence of strong microbial interactions between the causal organisms of eyespot, (*Oculimacula yallundae* and *O. acuformis*), and of brown foot rot, (*Microdochium nivale* and *Fusarium* spp.), previous reports on the development of stem-base diseases in cereals have indicated that there are positive associations between the different diseases and their pathogens. These associations have been discussed in Chapter 1.

Microdochium nivale and *Fusarium* spp. are considered to be often secondary colonisers of plant stem bases already infected by *Oculimacula* spp. (Bateman & Munnery, 1995). However, seed contaminated with *Fusarium* spp. or *M. nivale* often provides inoculum for seedling blight that in turn may be an alternative inoculum source for brown foot rot early in the season (Parry *et al.*, 1994; Humphreys *et al.*, 1995). Other factors influencing the availability of natural inoculum for the development of both brown foot rot and eyespot are different cultural practices. For example, ploughing has been shown to decrease brown foot rot incidence compared to non-inversion tillage (Prew *et al.*, 1995). Conversely, ploughing was reported in some instances to increase eyespot incidence and severity (Mielke, 1983; Murray *et al.*, 1991).

Bateman (1993) studied the development of stem-base diseases and interactions between their respective pathogens in three consecutive naturally infected crops of winter wheat. In a following study, Bateman & Munnery (1995) used a mixed mycelial inoculum of *M. nivale*, *Fusarium* spp. and *Oculimacula* spp. for the artificial inoculation of wheat plants at different times in controlled environment experiments. These studies indicated that *O. acuformis* was more tolerant than *O. yallundae* of secondary infections by *M.*

nivale and *Fusarium* spp. and suggested that this may be one of the factors contributing to the predominance of *O. acuformis* later in the crop's development. To further elucidate the associations between stem-base diseases and their pathogens, the present studies used wheat seed naturally infected with *M. nivale*, quantified at different DNA concentrations. Different cultivation practices were employed to create conditions more or less favourable for the development of brown foot rot and eyespot at different ranges of incidence or severity on which to investigate any relationships occurring between the *M. nivale* and *Oculimacula* spp. It was anticipated that ploughing would encourage the development of eyespot, whilst minimum cultivation treatments may favour more brown foot rot development.

The aims and objectives of this study were to use quantitative PCR assays to identify and study any relationships between the causal organisms of brown foot rot, *M. nivale* and eyespot, *O. acuformis* and *O. yallundae* throughout the crop's growing season and the development of brown foot rot or eyespot.

Null hypothesis tested: No relationships exist between *M. nivale* and *O. acuformis* or *O. yallundae* on stem bases of winter wheat.

MATERIALS AND METHODS

This study comprised two field experiments in which eyespot developed from natural inoculum.

Field experiment 1, cultivar Equinox.

Winter wheat, cultivar Equinox, was sown on 30 September 2002 as a second wheat on a field site at Harper Adams University College, Newport, Shropshire. All field operations carried out on the site are listed in Appendix 11. The experiment consisted of seven blocks

with three randomised cultivation treatments: ploughing, followed by power-harrowing and drilling (PPD); broadcasting seed onto stubble, followed by minimum tillage (BSM); and minimum tillage, followed by broadcasting seed (MBS). There were three plots in each block with dimensions of 3 x 24 m each. It was expected that different cultivation treatments before sowing a second winter wheat crop would provide different quantities of naturally-occurring eyespot and brown foot rot on which to determine any relationships between the stem-base pathogens.

Field experiment 2, cultivars Consort and Claire.

Winter wheat, cultivars Consort and Claire, were sown on 18 October 2002 as second wheat crops at a field site in Tibberton, Shropshire, previously used for an experiment in which a first winter wheat was inoculated with single isolates of *O. acuformis* and *O. yallundae* (Chapter 3). All field operations carried out on the site are described in Appendix 11. The experiment consisted of 10 randomised blocks with four treatments. These were commercially supplied Consort, treated with Beret Gold® (25g l^{-1} fludioxonil at application rate of 2 l t^{-1}), commercially supplied Claire, treated with Beret Gold® as above, untreated Consort naturally infected with *M. nivale* and Consort naturally infected with *M. nivale* and then treated in-house with Beret Gold® at the application rate mentioned above. Plot size was 6 m x 3 m.

To produce seed naturally infected with *M. nivale*, Consort was grown the previous year and inoculated at GS 65 with a spore suspension (total concentration of 10^5 spores $ml^{-1}$of water) of three isolates of *M. nivale* (30/1, 74/1, 117/1) supplied by Dr S. Edwards, Harper Adams University College, UK. This spore suspension was applied at 33 ml m^{-2} using a knapsack sprayer (Bastion 15, Application Technique Ltd, Herts, UK). Spore production involved sub-culturing each isolate by taking 5 mm diameter plugs of inoculum from the edges of actively growing cultures using a sterile cork borer and

transferring them onto plates of PDA. The cultures were initially incubated in darkness at 20°C for 14 days and then placed under continuous near-UV light for further 7-14 days at 20°C to induce sporulation. Conidial suspensions were obtained for each isolate by washing conidia from sporulating colonies using sterile distilled water. A sterile spatula was used to dislodge conidia and mycelium from the agar. The spore suspension was then filtered through two layers of sterile muslin to remove hyphal fragments and the obtained spore concentration was determined using a haemocytometer (Weber Scientific International Ltd, Teddington, Middlesex, UK) and adjusted to the above concentration. To achieve conditions conducive to ear blight development in the field, overhead mist-irrigation was applied on the crop for 60 seconds every 20 minutes between 08.00 and 18.00 hours. Mist irrigation continued for 21 days following inoculation. Subsequently the infected seed was harvested and used for the present experiment.

DNA of *M. nivale* was quantified in each seed batch before sowing in the field. Concentrations were 143.9, 210.7, 169.6 and 22.0 pg ng^{-1} of total DNA for untreated Consort, Consort treated in-house with Beret Gold®, commercially treated Consort and commercially treated Claire, respectively. At crop GS 23, plant counts per m^2 were carried out to determine any effects on the establishment of the crop.

Sampling and disease assessments

Thirty plants were collected from each plot at GS 23, 39 and 83 and assessed visually for eyespot and brown foot rot. Take-all was assessed at any growth stage when visual symptoms of the disease were observed on the wheat stem bases. Following visual assessment the stem bases were processed for DNA extraction and quantification.

Visual assessments of eyespot and brown foot rot incidence and DI in both experiments were carried out as described in Chapter 2. Take-all incidence was assessed as presence or absence of symptoms for each plant.

PCR quantification of fungal pathogens

DNA extraction and quantification from stems was described in Chapter 2. DNA extraction and quantification from wheat grain was the same as for stems, except 500 g of grain was milled in a Retsch DR100 mill (F. Kurt Retsch GmbH & Co, Germany) and 10 g from each milled sample was used for the extraction. Primers used and PCR conditions for quantitative assays for *O. acuformis* and *O. yallundae* are shown in Chapter 2. Primers, programmes and amplification conditions for *M. nivale* and *Fusarium* spp. are described in Chapter 4. DNA of *Gaeumannomyces graminis* var. *tritici* was quantified using primers and internal standards developed by Ruth Wilson, Harper Adams University College, UK.

Plots in Experiment 2 were harvested using a small-plot combine harvester (Winterstieger Crop Master), and grain yields (at 15% moisture content), thousand-grain weights and specific (hectolitre) weights were measured.

Statistical analyses

Statistical analysis using Genstat (Version 5 release 4.1, Lawes Agricultural Trust, IACR-Rothamsted, UK) was performed on all available data. Angular and log_{10} transformations were performed on some of the data for disease incidence/index and pathogen DNA, respectively, to obtain normal distributions required for analysis. Regression analysis was used where necessary to determine any relationship between variables.

RESULTS

Field experiment 1

Stem-base disease development and effects of treatments

Stem-base disease incidence and disease indices for each assessment are presented in Table 5.1a&b. At GS 23, no significant differences were observed between treatments for eyespot incidence or disease index. However, brown foot rot incidence was 32% higher in the ploughed plots (PPD) than in the plots where minimum tillage before (MBS) or after (BSM) broadcast seed application onto stubble were practised (Table 5.1a). At the same growth stage, the ploughed plots had 11% and 13% higher brown foot rot disease indices than plots with minimum tillage and seed application onto stubble, respectively (Table 5.1b). There were no significant differences between treatments in incidence or disease index of eyespot, brown foot rot or take-all at GS 39 or GS 83. Brown foot rot incidence and disease index steadily decreased between GS 23 and GS 83. In contrast, eyespot incidence and disease index increased throughout the growing season in all treatments and at GS 83 highest levels were observed in the ploughed plots. Take-all incidence on the stem bases was observed first at GS 39, and at GS 83 take-all incidence reached more than 30% in the ploughed plots.

Stem-base pathogen development and effects of treatments

Microdochium nivale was the dominant pathogen present on the stem-bases at all growth stages. There were no significant differences in pathogen DNA quantified at any growth stage. Mean amounts of pathogen DNA of *Oculimacula* spp. and *M. nivale* are presented in Table 5.2.

In contrast to brown foot rot incidence and disease index, DNA of *M. nivale* increased rapidly between GS 23 and GS 39 with about a 50-fold increase in the minimum cultivation plots but remained almost unchanged between GS 39 and GS 83 (Fig. 5.3a). Conversely, DNA concentrations of *O. acuformis* and *O. yallundae* increased more between GS 39 and GS 83 (Fig. 5.3a).

Relationships between visually assessed diseases and pathogen DNA

A significant positive relationship was observed between disease indices of eyespot and brown foot rot at GS 39 (Table 5.3). Eyespot incidence at GS 39 also correlated positively with eyespot disease index at GS 83, accounting for 27% of the variance. Using DNA concentrations of *M. nivale* or *O. yallundae* at GS 23 to predict eyespot disease index at GS 83 indicated a significant but weak correlation (Table 5.3).

Relationships between stem-base pathogens

Regression analysis revealed significant relationships between DNA concentrations of *M. nivale*, *O. acuformis* and *O. yallundae* at each growth stage (Figure 5.1). Analyses of position and parallelism showed that at each growth stage DNA of *M. nivale* and *O. acuformis* (Figure 5.1a) or *O. yallundae* (Figure 5.1b) fitted positive parallel lines. Conversely, the data for the DNA concentrations of the two *Oculimacula* spp. was fitted best by a common line for the different growth stages and the regression accounted for more than 70% of the variance (Figure 5.1c).

Table 5.1a. Incidence of brown foot rot, eyespot and take-all at each growth stage in experiment 1.

	Angular transformed disease incidence % (back-transformed values in parentheses)							
	GS 23			GS 39			GS 83	
Treatment	E[a]	BFR[b]	E	BFR	TA[c]	E	BFR	TA
MBS[d]	31.6 (27.5)	62.9(79.3)	42.0(44.8)	58.0(72.0)	26.8(20.3)	54.9(66.9)	58.7(73.0)	19.9(11.6)
BSM[e]	25.9(19.1)	62.9(79.3)	42.5(45.6)	57.2(70.7)	12.2(4.5)	53.5(64.6)	53.0(63.8)	24.6(17.3)
PPD[f]	25.0(17.9)	74.3(92.7)	43.3(47.0)	65.6(82.9)	22.1(14.2)	56.9(70.2)	56.2(69.1)	36.9(36.1)
CV	42.2	11.9	26.3	12.5	69.7	18.0	11.8	52.1
SED	6.2	4.3	6.0	4.0	7.8	5.3	3.5	7.6
LSD	13.5	9.3	13.1	8.8	16.5	11.5	7.7	16.5
P	0.534	0.029	0.976	0.113	0.185	0.818	0.310	0.108

[a] eyespot, [b] brown foot rot, [c] take-all, [d] minimum tillage, followed by broadcast seed application, [e] broadcast seed application onto stubble, followed by minimum tillage, [f] ploughing, followed by power harrow and drill

Table 5.1b. Disease index for brown foot rot, eyespot and take-all at each growth stage in experiment 1.

	Angular transformed disease index % (back-transformed values in parentheses)					
	GS 23		GS 39		GS 83	
Treatment	E[a]	BFR[b]	E	BFR	E	BFR
MBS[c]	27.4(21.2)	39.8(40.9)	34.5(32.1)	33.1(29.9)	45.6(51.1)	34.4(32.0)
BSM[d]	22.2(14.3)	38.8(39.2)	33.5(30.5)	34.0(31.2)	46.3(52.3)	31.4(27.2)
PPD[e]	22.6(14.8)	44.7(49.5)	35.7(34.1)	37.2(36.5)	52.1(62.3)	33.1(29.8)
CV	44.1	10.0	28.6	12.5	17.3	11.5
SED	5.7	2.2	5.3	2.3	4.4	2.0
LSD	12.3	4.8	11.5	5.1	9.7	4.4
P	0.611	0.041	0.917	0.226	0.305	0.369

[a] eyespot, [b] brown foot rot, [c] minimum tillage, followed by broadcast seed application, [d] broadcast seed application onto stubble, followed by minimum tillage, [e] ploughing, followed by power harrow and drill

Table 5.2. Mean concentration of DNA of *Oculimacula acuformis*, *O. yallundae* and *Microdochium nivale* at each growth stage in experiment 1.

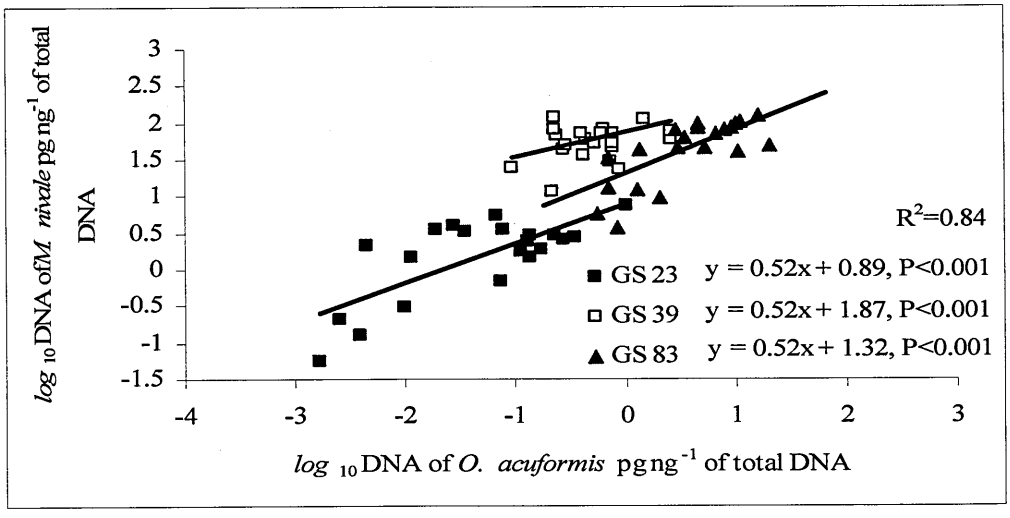
	log $_{10}$ pathogen DNA pg ng^{-1} of total DNA								
	GS 23			GS 39			GS 83		
Treatment	*O. a*[a]	*O. y*[b]	*M. n*[c]	*O. a*	*O. y*	*M. n*	*O. a*	*O. y*	*M. n*
MBS	-1.60(0.03)	-0.83(0.15)	0.28(1.91)	-0.16(0.69)	0.26(1.82)	1.78(60.26)	0.45(2.82)	1.25(17.78)	1.47(29.51)
BSM	-1.30(0.05)	-0.44(0.36)	0.41(2.57)	-0.50(0.32)	0.06(1.15)	1.58(38.02)	0.49(3.09)	1.26(18.20)	1.57(37.15)
PPD	-1.13(0.07)	-0.53(0.30)	0.39(2.45)	-0.21(0.62)	0.19(1.55)	1.79(61.66)	0.74(5.50)	1.59(38.90)	1.79(61.66)
SED	0.32	0.26	0.23	0.20	0.16	0.14	0.27	0.27	0.25

[a] *Oculimacula acuformis*, [b] *Oculimacula yallundae*, [c] *Microdochium nivale*

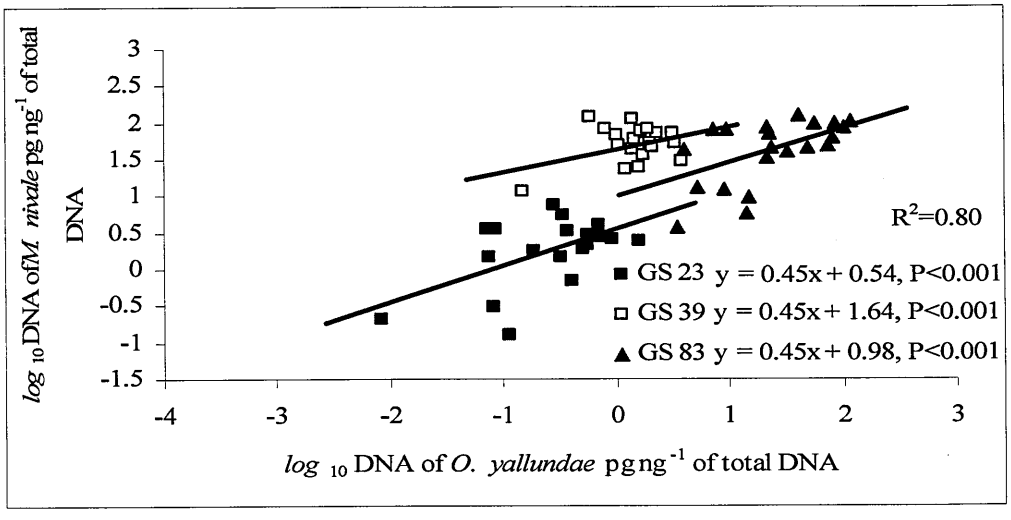
Table 5.3. Summary of significant regressions for experiment 1.

Response variate	Explanatory variate	Regression equation	% Variance accounted for	P
AFDI[a] at GS 39	AEDI at GS 39	y = 0.29x + 24.8	26	0.01
AEDI[b] at GS 83	AEI[c] at GS 39	y = 0.50x + 26.5	27	0.01
AEDI at GS 83	*log* $_{10}$ DNA of *M. nivale* at GS 23	y = 7.09x + 46.8	18	0.03
AEDI at GS 83	*log* $_{10}$ DNA of *O. yallundae* at GS 23	y = 7.54x + 52.7	17	0.04

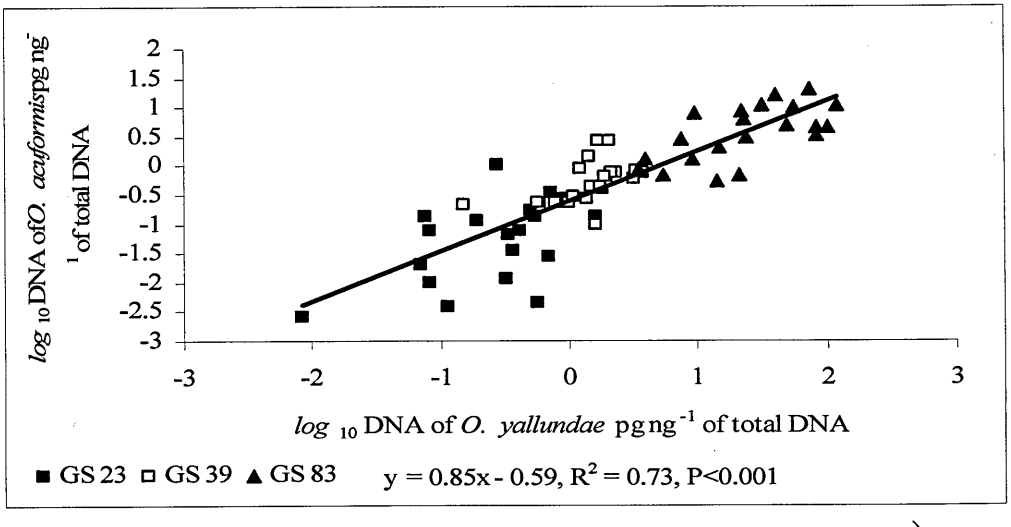
[a] angular transformed brown foot rot disease index, [b] angular transformed eyespot disease index, [c] angular transformed eyespot incidence



a)



b)



c)

Figure 5.1. Regressions of DNA of *M. nivale* on DNA of *O. acuformis* (a) and *O. yallundae* (b), and of DNA of *O. acuformis* on DNA of *O. yallundae* (c) for all growth stages of cv. Equinox, Experiment 1.

Field experiment 2

Stem-base disease development and effects of treatments

Significant differences between treatments in brown foot rot and eyespot incidence and disease index were found at GS 23 (Table 5.4a&b). At GS 23, commercially supplied Consort had significantly lower eyespot incidence and disease index than any of the other treatments. At the same growth stage, both Claire and commercially supplied Consort also had significantly lower brown foot rot incidence and disease index compared to the rest of the treatments. At GS 39, brown foot rot incidence and disease index were significantly higher in Claire than any of the other treatments. Although no significant differences were observed for eyespot incidence at GS 39, eyespot disease index in Claire was more than 23% higher than in the untreated Consort and commercially supplied Consort. At GS 83, although the lowest brown foot rot incidence was recorded in Claire, no significant differences were observed between treatments for brown foot rot disease index and eyespot incidence and disease index (Table 5.4a&b). Take-all incidence on the stem-bases was first observed at GS 83 and no significant differences were found between treatments. Eyespot incidence and disease index increased steadily throughout the growing season. Similarly, brown foot rot disease index also increased, although brown foot rot incidence decreased slightly in Claire from GS 39 to GS 83.

Stem-base pathogen development and effects of treatments

DNA of *M. nivale* quantified at GS 23 was found in relatively higher concentration than DNA of either *O. acuformis* or *O. yallundae* in all treatments. Differences between species were not large, however, indicating no apparent predominance of one of the stem-base pathogens at this time (Table 5.5). Furthermore, in the in-house treated Consort, in

which *M. nivale* in seed was found to be at the highest concentration (> 200 pg ng $^{-1}$ of total DNA), the combined DNA concentration of both *Oculimacula* spp. on the stem-bases exceeded that of *M. nivale* by more than 20%. At GS 23, significant differences between treatments were found only for DNA of *O. acuformis* (Table 5.5). DNA concentration of *O. acuformis* found in the in-house treated Consort was significantly higher than in any of the other treatments. At GS 39, commercially supplied Consort had significantly lower concentration of *O. acuformis* DNA compared to any other treatment, signifanctly lower concentration of *M. nivale* DNA compared to the untreated Consort and significantly lower concentration of *O. yallundae* DNA compared to Claire. No signficant differences were observed between treatments for DNA concentration of *O. acuformis* and *M. nivale* at GS 83. However, at the same growth stage, DNA concentration of *O. yallundae* was significantly higher in Claire than in any of the other treatments. Overall DNA concentration of all three species increased on average more than 60-fold between GS 23 and GS 39 whilst smaller DNA increases of average of 3-fold on average were observed between GS 39 and GS 83 (Fig. 5.3b).

There were no significant differences between treatments for DNA concentrations of *Gaeumannomyces graminis* var. *tritici* at GS 39 and GS 83, or *Fusarium* spp. at GS 83 (Table 5.6). PCR quantification of *M. nivale* and *Fusarium* spp. in the grain at GS 92 also failed to reveal any significant differences between treatments (Table 5.6).

Relationships between visually assessed diseases and pathogen DNA

Regression analyses revealed significant but weak relationships between pathogen DNA and the respective disease incidence or index on most occasions (Table 5.8). However, at the early growth stages, significant but weak relationships were also found between DNA of *M. nivale* and eyespot disease index and between DNA of *O. yallundae* and brown foot rot disease index (Table 5.8). Significant but weak relationships were also found between

DNA of *M. nivale* and brown foot rot disease index at GS 23 and DNA of *M. nivale* present in the grain at GS 92 (Table 5.8).

Relationships between stem-base pathogens

DNA of *O. yallundae* at GS 23 and GS 39 was correlated to DNA concentration at GS 83, but no such relationship was present for DNA concentration of *O. acuformis* (Table 5.8). DNA of *Gaeumannomyces graminis* var. *tritici* correlated positively with DNA concentrations of *M. nivale* and *Fusarium* spp. and negatively with DNA concentrations of both *Oculimacula* spp. at GS 83, with the regression accounting for 38% of the variance.

Analysis of position and parallelism revealed that the DNA data of *M. nivale* and *O. acuformis* were best fitted by positive parallel regression lines for each growth stage (Figure 5.2a). In contrast, the data for DNA concentrations of *M. nivale* and *O. yallundae* were best fitted by separate, non-parallel lines for each growth stage (Figure 5.2b). The data for the DNA concentrations of the two *Oculimacula* spp. throughout the growing season were best fitted by positive parallel lines for each growth stage (Figure 5.2c).

Grain yield and yield components

Significant differences between treatments were found for plant numbers at GS 23, grain yield, thousand-grain weight and specific weight (Table 5.7). Untreated Consort and commercially supplied Consort had significantly lower plant numbers m^{-2} at GS 23 than Claire and in-house treated Consort. Commercially supplied Consort also had 18% lower yield than any other treatment. Significantly lower thousand-grain weights and specific weights were measured for Claire and commercially supplied Consort than for the rest of the treatments (Table 5.7). Losses in grain yield, thousand-grain weight, and specific weight were associated mainly with take-all incidence at GS 83, accounting for 40%

(Table 5.8), 40% and 43% of the variance, respectively. A negative relationship between yield and DNA of *Gaeumannomyces graminis* var. *tritici* at GS 83 was also found (Table 5.8). The regression, however accounted for only 24% of the variance indicating that DNA concentration of *G. graminis* var. *tritici* in the stem bases was less reliable in predicting yield loss.

DNA accumulation over the growing season and multiplication factor for changes in the DNA concentration between growth stages for each pathogen in both experiments are shown in Fig. 5.3.

Table 5.4a. Incidence of brown foot rot, eyespot and take-all at each growth stage in experiment 2.

	Angular transformed disease incidence % (back-transformed values in parentheses)						
	GS 23		GS 39		GS 83		
Treatment	E[a]	BFR[b]	E	BFR	E	BFR	TA[c]
U Consort[d]	33.5(30.5)	33.2(30.0)	38.4(38.6)	33.7(30.8)	63.0(79.4)	42.9(46.32)	23.4(15.8)
T Consort[e]	35.9(34.4)	30.6(25.9)	39.9(41.2)	32.6(29.1)	64.8(91.9)	48.1(55.4)	20.4(12.2)
C Consort[f]	24.6(17.3)	14.9(6.6)	38.5(38.8)	31.9(28.0)	56.0(68.7)	43.8(48.0)	30.3(25.5)
Claire[g]	35.2(33.2)	17.8(9.3)	44.4(49.0)	41.3(43.5)	59.0(74.5)	37.1(36.3)	29.2(23.8)
CV	28.0	46.1	17.4	17.8	17.7	11.9	34.0
SED	4.0	5.0	3.1	2.8	4.8	2.3	3.9
LSD	8.3	10.2	6.5	5.7	9.9	4.7	8.1
P	0.034	0.002	0.212	0.008	0.282	<0.001	0.054

[a] eyespot, [b] brown foot rot, [c] take-all, [d] Consort seed, infected with *M. nivale*, [e] Consort seed infected with *M. nivale* and treated in-house with 25g l^{-1} fludioxonil at application rate of 2 l t^{-1}, [f] Commercially available Consort, treated with 25g l^{-1} fludioxonil at application rate of 2 l t^{-1}, [g] Commercially available Claire, treated with 25g l^{-1} fludioxonil at application rate of 2 l t^{-1}

Table 5.4b. Disease index for brown foot rot, eyespot and take-all at each growth stage in experiment 2.

	Angular transformed disease index % (back-transformed values in parentheses)					
	GS 23		GS 39		GS 83	
Treatment	E [a]	BFR [b]	E	BFR	E	BFR
U Consort[c]	18.8(10.3)	18.1(9.6)	26.8(20.3)	20.4(12.1)	48.6(56.3)	33.4(30.3)
T Consort[d]	19.9(11.6)	16.4(8.0)	27.8(21.8)	20.4(12.1)	52.2(62.4)	37.3(36.7)
C Consort[e]	13.8(5.7)	8.4(2.1)	25.1(18.0)	18.4(10.0)	48.1(55.4)	35.4(33.5)
Claire [f]	19.8(11.4)	10.2(3.1)	32.8(29.3)	24.4(17.0)	52.5(62.9)	32.7(29.2)
CV	26.4	43.3	20.3	21.8	14.1	18.2
SED	2.1	2.6	2.6	2.0	3.2	2.8
LSD	4.4	5.3	5.3	4.2	6.5	5.8
P	0.023	0.002	0.035	0.046	0.393	0.375

[a]eyespot, [b]brown foot rot, [c]Consort seed, infected with *M. nivale*, [d]Consort seed infected with *M. nivale* and treated in-house with 25g l^{-1} fludioxonil at application rate of 2 l t^{-1}, [e]Commercially supplied Consort, treated with 25g l^{-1} fludioxonil at application rate of 2 l t^{-1}, [f]Commercially supplied Claire, treated with 25g l^{-1} fludioxonil at application rate of 2 l t^{-1}

Table 5.5. Pathogen DNA of *Oculimacula acuformis*, *O. yallundae* and *M. nivale* at each growth stage in experiment 2.

	log $_{10}$ pathogen DNA pg ng^{-1} of total DNA (back-transformed values in parentheses)								
	GS 23			GS 39			GS 83		
Treatment	*O. a*[a]	*O. y*[b]	*M. n*[c]	*O. a*	*O. y*	*M. n*	*O. a*	*O. y*	*M. n*
U Consort[d]	-0.83(0.15)	-1.12(0.08)	-0.36(0.44)	0.44(2.72)	0.96(9.14)	1.62(41.69)	0.85(7.13)	1.17(14.93)	1.92(82.79)
T Consort[e]	-0.54(0.29)	-0.61(0.24)	-0.39(0.41)	0.46(2.88)	0.88(7.66)	1.48(30.06)	0.95(8.99)	1.27(18.49)	1.98(95.50)
C Consort[f]	-1.15(0.07)	-0.99(0.10)	-0.58(0.27)	0.07(1.16)	0.65(4.45)	1.37(23.66)	0.79(6.18)	1.27(18.41)	2.09(121.90)
Claire[g]	-0.95(0.11)	-0.77(0.17)	-0.75(0.18)	0.33(2.15)	1.43(27.10)	1.49(31.19)	0.88(7.57)	1.92(82.60)	1.85(70.96)
CV	42.5	57.9	99.5	63.8	56.5	10.7	29.9	28.4	17.4
SED	0.2	0.2	0.2	0.1	0.3	0.1	0.1	0.2	0.2
LSD	0.3	0.5	0.5	0.2	0.5	0.2	0.2	0.4	0.3
P	0.008	0.138	0.317	<0.001	0.028	0.018	0.576	<0.001	0.475

[a] *Oculimacula acuformis*, [b] *Oculimacula yallundae*, [c] *Microdochium nivale*, [d] Consort seed, infected with *M. nivale*, [e] Consort seed infected with *M. nivale* and treated in-house with 25g l^{-1} fludioxonil at application rate of 2 l t^{-1}, [f] Commercially supplied Consort, treated with 25g l^{-1} fludioxonil at application rate of 2 l t^{-1}, [g]Commercially supplied Claire, treated with 25g l^{-1} fludioxonil at application rate of 2 l t^{-1}

Table 5.6. Mean pathogen DNA of *Fusarium* spp. and *Gaeumannomyces graminis* var. *tritici* in stems at GS 83, and *Fusarium* spp. and *M. nivale* found in wheat grain at harvest in experiment 2.

	Pathogen DNA pg ng^{-1} of total DNA					
	GS 39		GS 83		GS 92 wheat grain	
Treatment	*F*[a]	*Ggt*[b]	*F*	*Ggt*	*M. n*[c]	*F*
U Consort[d]	-	0.66	6.86	41.0	0.50	2.54
T Consort[e]	-	1.04	1.01	63.0	0.70	1.71
C Consort[f]	-	0.05	6.27	88.0	0.44	2.64
Claire[g]	-	0.59	4.41	55.0	0.61	2.31
SED	-	0.9	2.5	28.6	0.2	0.4

[a] *Fusarium* spp., [b] *Gaeumannomyces graminis* var. *tritici*, [c] *Microdochium nivale*, [d] Consort seed, infected with *M. nivale*, [e] Consort seed infected with *M. nivale* and treated in-house with 25g l^{-1} fludioxonil at application rate of 2 l t^{-1}, [f] Commercially supplied Consort, treated with 25g l^{-1} fludioxonil at application rate of 2 l t^{-1}, [g] Commercially supplied Claire, treated with 25g l^{-1} fludioxonil at application rate of 2 l t^{-1}

- not detected by PCR assay

Table 5.7. Plant numbers at GS 23, grain yield, specific weight and thousand-grain weight in experiment 2.

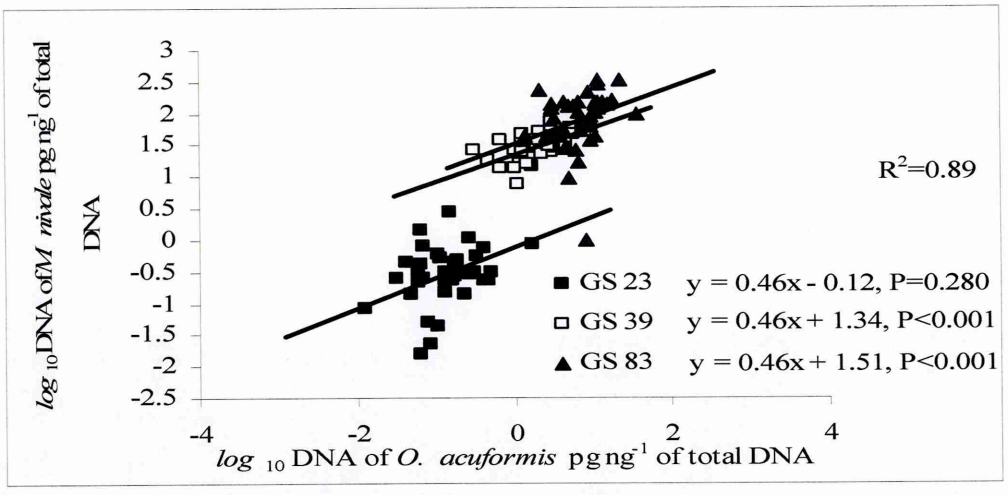
Treatment	Plant number m^{-2}	Yield t ha^{-1}	Specific weight kg hl^{-1}	TGW g
U Consort[a]	190.4	3.8	66.5	32.3
T Consort[b]	213.6	3.8	66.6	31.8
C Consort[c]	183.2	3.1	64.4	29.6
Claire[d]	223.2	3.8	63.3	27.7
CV	14.0	14.7	3.0	4.8
SED	12.7	0.2	0.9	0.7
LSD	26.1	0.5	1.8	1.3
P	0.012	0.009	<0.001	<0.001

[a] Consort seed, infected with *M. nivale*, [b] Consort seed infected with *M. nivale* and treated in-house with 25g l^{-1} fludioxonil at application rate of 2 l t^{-1}, [c] Commercially supplied Consort, treated with 25g l^{-1} fludioxonil at application rate of 2 l t^{-1}, [d] Commercially supplied Claire, treated with 25g l^{-1} fludioxonil at application rate of 2 l t^{-1}

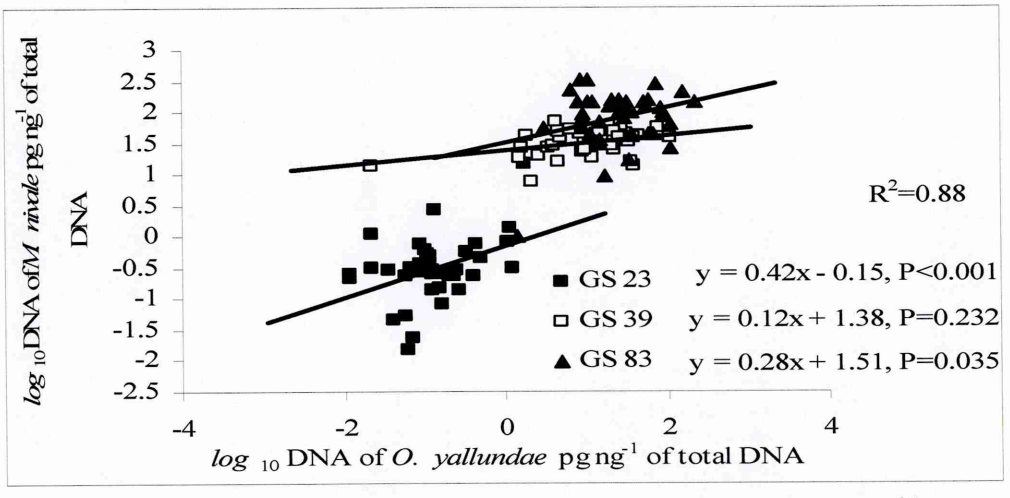
Table 5.8. Summary of significant regressions for experiment 2.

Response variate	Independent variate	Regression equation	% Variance accounted for	P
ABFDI[a] at GS 23	*log* $_{10}$ DNA of *M. nivale* at GS 23	y = 7.8x + 17.3	19	0.003
ABFDI at GS 39	*log* $_{10}$ DNA of *M. nivale* at GS 39	y = 8.2x + 8.6	11	0.024
ABFDI at GS 39	*log* $_{10}$ DNA of *O. yallundae* at GS 39	y = 2.6x + 18.4	10	0.028
AEDI[b] at GS 39	*log* $_{10}$ DNA of *M. nivale* at GS 23	y = 4.0x + 30.2	9	0.031
AEDI at GS 39	*log* $_{10}$ DNA of *O. yallundae* at GS 23	y = 4.1x + 31.6	10	0.028
log $_{10}$ DNA of *O. acuformis* at GS 39	AEDI at GS 23	y = 0.02x - 0.03	19	0.003
log $_{10}$ DNA of *O. yallundae* at GS 39	AEDI at GS 23	y = 0.04x + 0.3	11	0.022
log $_{10}$ DNA of *O. yallundae* at GS 83	AEDI at GS 39	y = 0.04x + 0.4	21	0.002
AEDI at GS 83	*log* $_{10}$ DNA of *O. acuformis* at GS 83	y = 13.9x + 38.2	23	<0.001
TAI[c] at GS 83	*log* $_{10}$ DNA of *G. graminis* var. *tritici* at GS 83	y = 23.4x - 18.1	16	0.035
log $_{10}$ DNA of *M. nivale* in grain	ABFDI at GS 23	y = 0.01x – 0.5	14	0.011
log $_{10}$ DNA of *M. nivale* in grain	*log* $_{10}$ DNA of *M. nivale* at GS 23	y = 0.21x – 0.2	13	0.011
log $_{10}$ DNA of *O. yallundae* at GS 83	*log* $_{10}$ DNA of *O. yallundae* at GS 39	y = 0.34x + 1.1	19	0.003
log $_{10}$ DNA of *O. yallundae* at GS 83	*log* $_{10}$ DNA of *O. yallundae* at GS 23	y = 0.44x + 1.8	20	0.002
log $_{10}$ DNA of *G. graminis* var. *tritici* at GS 83	*log* $_{10}$ DNA of *M. nivale* at GS 83	y = 0.59x + 0.5	23	0.002
Yield t ha^{-1}	TAI at GS 83	y = 4.2 - 0.03x	40	<0.001
Yield t ha^{-1}	*log* $_{10}$ DNA of *G. graminis* var. *tritici* at GS 83	y = 5.4 – 1.1x	24	0.002

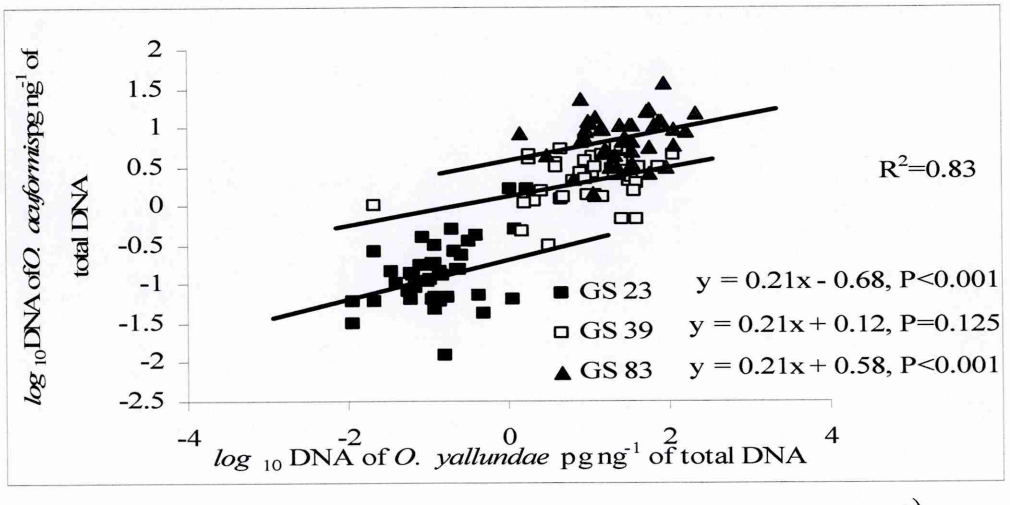
[a] angular transformed brown foot rot disease index, [b] angular transformed eyespot disease index, [c] take-all incidence



a)

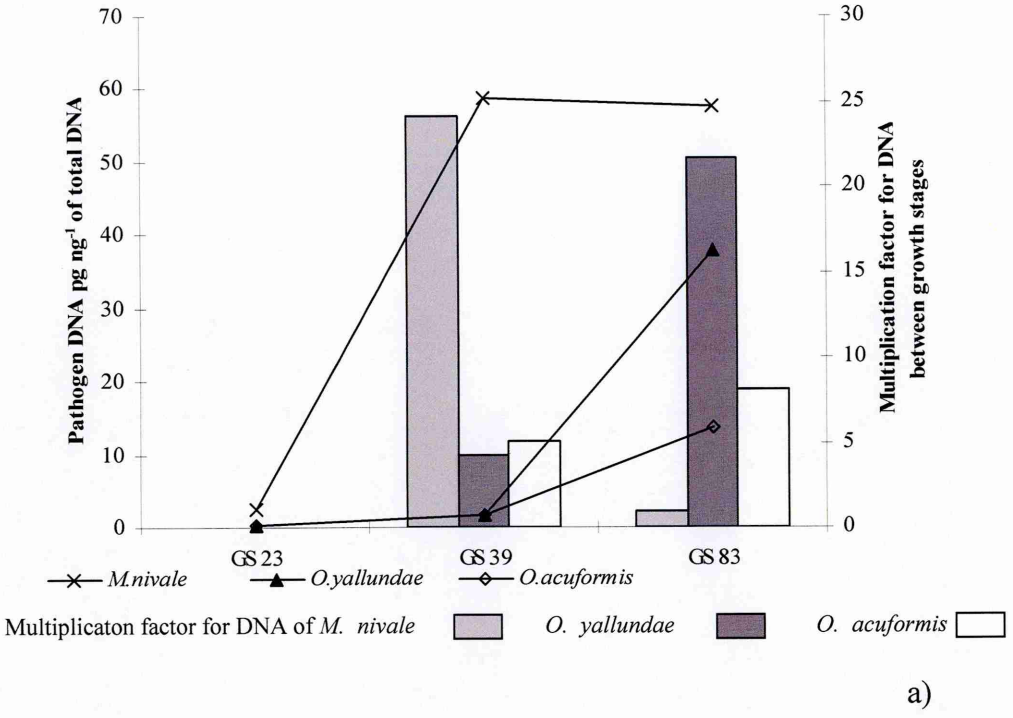


b)

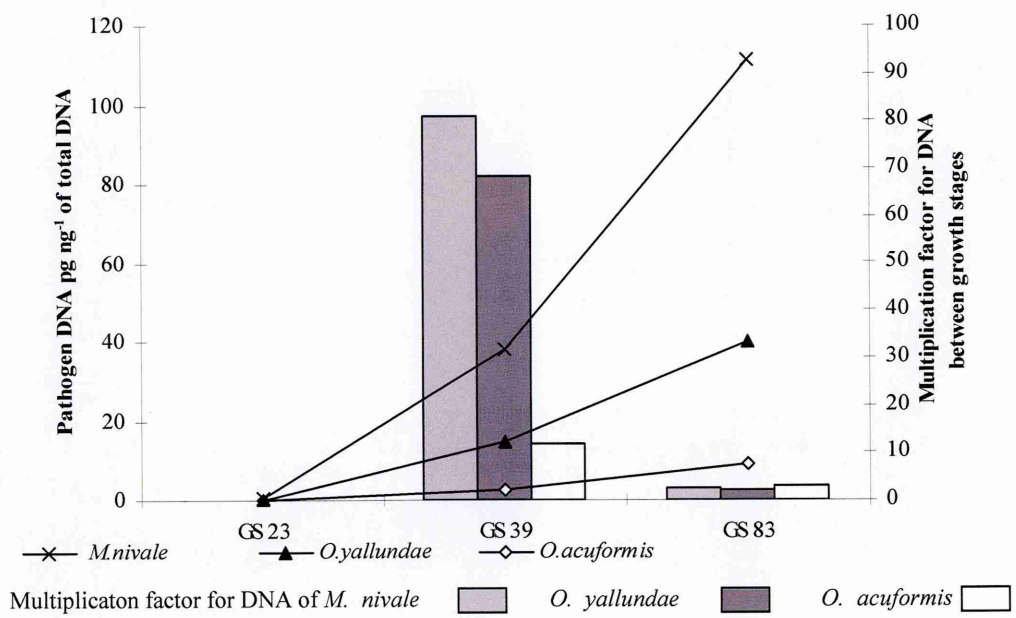


c)

Figure 5.2. Regressions of DNA of *M. nivale* on DNA of *O. acuformis* (a) and *O. yallundae* (b), and of DNA of *O. acuformis* on DNA of *O. yallundae* (c) for all growth stages of cvs. Claire and Consort, Experiment 2.



a)



b)

Figure 5.3. Pathogen DNA accumulation over the growing season and multiplication factor for changes in DNA (averaged over all treatments) between crop growth stages for field experiment 1(a) and 2 (b).

DISCUSSION

Published research on the effects of cultural practices on the incidence and severity of stem-base diseases has shown that ploughing the soil in the cereal rotation usually decreases brown foot rot but it has more variable effects on eyespot (Jenkyn *et al.*, 1994; Prew *et al.*, 1995). The first field experiment, however, showed that at GS 23, brown foot rot incidence and disease index were significantly higher in the ploughed plots than in the non-inversion plots. Nevertheless, this effect was not consistent throughout the growing season as indicated by the absence of significant differences between treatments for brown foot rot assessments at GS 39 and GS 83. Visual assessments of eyespot and take-all at any of the growth stages also failed to reveal any differences between cultivation practices, thus confirming reports on the inconsistent and largely variable effects of cultivation methods on these diseases (Yarham & Norton, 1981; Jenkyn *et al.*, 1994; Hornby *et al.*, 1998).

The absence of significant effects of cultivation on eyespot and take-all can often be attributed to other additional factors. For example, although ploughing buries most of the infectious debris left from the previous crop, thus decreasing available inoculum, there is evidence that only a small amount of inoculum is required for the initiation of eyespot infection (Hollins & Scott, 1980). In addition, subsequent disease development has been shown to be affected more by favourable environmental conditions than initial inoculum levels (Colbach *et al.*, 1997). Ploughing may also return debris from earlier crops, on which eyespot pathogens are still surviving (Macer, 1961a & b), to the surface. Therefore, it is not suprising that in many experiments ploughing has shown little effect on eyespot disease. Conversely, effects on take-all may be influenced largely by soil management and quality of cultivation rather than the choice of cultivation practice (Yarham & Norton, 1981). It should be noted, however, that one year of experimentation, as in the first field

experiment presented here, is not sufficient to provide any conclusive results on the effects of cultivation treatments and longer term experiments are required for this purpose. Furthermore, the main objective of this experiment was to provide different incidences or severities of the stem-base diseases on which to investigate possible relationships between their respective pathogens.

In the second experiment, although the commercially supplied Consort, and Claire treated with fludioxonil, had less brown foot rot than the naturally infected Consort and Consort treated in-house, and initial *M. nivale* DNA in the seed was also different, no significant differences were observed for the DNA of *M. nivale* at GS 23. However, DNA of *M. nivale* var. *majus* and var. *nivale* were quantified together here. Further information may have been obtained by quantifying their DNA separately. This may also has contributed to information on interactions between *M. nivale* and *Oculimacula* spp. Glynn (2002) showed that isolates of *M. nivale* var. *nivale* exhibit less sensitivity towards fludioxinil than isolates of var. *majus*.

Plant numbers at GS 23 failed to correlate with the amount of DNA of *M. nivale* in the stem bases. When considered with the lack of significant effects of seed treatment on the DNA concentrations of *M. nivale* in plants, this is further indication that seedling blight failed to develop sufficiently to cause reductions in plant numbers or to contribute to brown foot rot in the second experiment. Since the ability of *M. nivale* to inhibit seed germination and to reduce plant numbers has been shown to be favoured by lower air temperatures (Glynn, 2002) and cold, dry soil (Millar & Colhoun, 1969) it is possible that environmental conditions at the early seedling stages of the plants were not conducive to the development of the seedling blight phase of disease.

Eyespot development proceeded similarly in both experiments, disease incidence and index increasing steadily throughout the growing season. In contrast, brown foot rot showed a different pattern of development in the two experiments. In the first experiment, brown foot rot incidence and disease index decreased from GS 23 to GS 39 and remained

almost unchanged at GS 83. A similar decline has been observed by others (Parry, 1990; Nicholson *et al.*, 2002; Turner *et al.*, 2002) and on most occasions was associated with the natural death of large numbers of late-formed tillers accompanied by the death of stem-base leaf sheaths. However, the observed decline in brown foot rot incidence and disease index was not in agreement with the DNA quantification of *M. nivale*, which decreased only in the minimum cultivation plots after GS 39. Therefore, it is likely that either the symptoms of the brown foot rot were less dependent on pathogen DNA, as indicated by the variable relationship between visual assessment and PCR quantification data, or some mis-indentification of brown foot rot and eyespot symptoms occurred at GS 39 and GS 83. Indeed, the significant positive relationship between visually assessed brown foot rot disease index and eyespot disease index at GS 39 supports this statement. In the second experiment, brown foot rot generally followed the same pattern of development as eyespot and, although both diseases were at similar levels at GS 23 and GS 39, eyespot incidence and disease index were 30% higher than brown foot rot by GS 83.

In the first experiment, incidence and disease index of brown foot rot were twice as high as those of eyespot at GS 23. In contrast, in the second exeriment, in which seed infected with different amounts of *M. nivale* was sown, assessments at GS 23 showed that the incidence and disease index of brown foot rot were similar to or less than those of eyespot. DNA quantification of stem-base pathogens in both experiments generally confirmed visual assessments. Thus, at GS 23, DNA of *M. nivale* in the first experiment was about five times more than DNA of *Oculimacula* spp. In comparison, in the second experiment, differences between DNA concentrations of *M. nivale* and *Oculimacula* spp. were negligable.

These observations, although limited because of the lack of data for DNA concentration of *M. nivale* present in the seed in the first experiment, impose questions regarding the significance of seed-borne inoculum of *M. nivale* for the development of brown foot rot. Indeed, there was a poor correlation between DNA concentration of *M.*

nivale quantified in the seed and in stem-bases at GS 23 or between DNA of *M. nivale* in the seed and brown foot rot incidence at GS 23. In addition, the DNA concentration of *M. nivale* present in the untreated seed used in this study was four times higher than the average concentration of this pathogen detected in the untreated seed used in other seedling blight experiments (Glynn, 2002) indicating the presence of large amounts of available inoculum. Furthermore, the absence of large increases in brown foot rot incidence and disease index at GS 23 in this experiment, where high concentrations of DNA of *M. nivale* were present in the seed, suggests that seed-borne *M. nivale* may not be an important inoculum source for brown foot rot or that its effectiveness may have been hampered by less favourable environmental conditions.

Previous reports (Nicholson *et al.*, 2002; Turner *et al.*, 2002) have shown that the time immediately following stem extension in cereals is the period when the largest DNA concentrations of *M. nivale* are detected. Similarly, in the present studies, the largest increases in DNA concentration of *M. nivale* of more than 20-fold in the first experiment and more than 90-fold in the second experiment were observed between GS 23 and GS 39, indicating that this is the main period of accumulation of *M. nivale* in the stem bases. Although even larger amounts of *M. nivale* were generally detected at GS 83, the actual increases in pathogen DNA between GS 39 and GS 83 were less. Thus, the average increase in DNA of *M. nivale* between GS 39 and GS 83 was less than 3-fold in the second experiment, whilst in the first experiment DNA concentrations stayed unchanged. In contrast, the DNA increases of both *Oculimacula* spp. took place at different times in the two experiments. In the first experiment, DNA concentrations of both *Oculimacula* spp. increased more dramatically after GS 39. In the second experiment, DNA of both *Oculimacula* spp. increased more than 10-fold between GS 23 and GS 39, compared with an average of 3-fold between GS 39 and GS 83. The main difference between the two experiments regarding the stem-base pathogen population was at GS 23 when, in the first experiment, there was clear predominance of *M. nivale* with DNA concentrations at least

five times higher than those of the two *Oculimacula* spp. In contrast, in the second experiment differences between concentrations of *M. nivale* and *Oculimacula* spp. at GS 23 were less. Although the sequence of infection by the stem-base pathogens in both experiments is unclear, the initial predominance of *M. nivale* in the first experiment indicates that infections by this pathogen may have occurred earlier than infection by *Oculimacula* spp., which may have provided some competitive advantage to the former to become established and colonise the stem bases earlier. This is supported by the higher incidence and disease index of brown foot rot at GS 23. In the second experiment, where the difference in the DNA concentration of the different pathogens was less, all species increased their respective DNA concentrations earlier, prior to GS 39. Nevertheless, the predominance of *M. nivale* in the stem bases of plants in the second experiment at GS 83 suggests that other factors, for example environmental conditions, are probably also important for the late development of the stem-base pathogens.

Both *Oculimacula* spp. were present on the stem bases in quantifiable amounts as early as GS 23, indicating that infection by both species occurred at similar times. Others (King & Griffin, 1985; Coşkun *et al.*, 1987; Nicholson *et al.*, 2002) have noticed that *O. yallundae* was more common than *O. acuformis* in the early plant samples up to GS 30. Of the two *Oculimacula* spp., *O. yallundae* was always found at higher DNA concentrations at all growth stage in both experiments. DNA of both species increased at the same times, although DNA of *O. yallundae* increased more in both experiments. Greater increase in DNA of *O. yallundae* than of *O. acuformis* late in the growing season, between GS 60 and GS 72 was also observed in other experiments (Chapter 3) and was associated with relative reductions in plant DNA, most likely due to increased lignification and cell wall thickening as a reaction to eyespot infection (Murray & Bruehl, 1983). However, DNA increases in the present studies were not as large as those described in Chapter 3 and no clear correlation was established with total DNA in the plant samples. In both experiments, DNA of *O. acuformis* failed to accumulate in amounts comparable to

those of DNA of *O. yallundae*. Although there is unsufficient evidence (Goulds & Fitt, 1990a; Bateman *et al.*, 1990a) that the two species are affected differently by environmental conditions, it is possible that the occurrence of dry weather during the period of stem extension, by increasing the rate of death of leaf sheaths and impairing stem lesion establishment (Higgins *et al.*, 1986) may have affected the slower growing *O. acuformis* more than *O. yallundae* or *M. nivale*.

In the first experiment, the symptoms of stem-base diseases failed to correlate with their respective pathogens. In the second experiment, relationships between visual symptoms and DNA concentrations at each growth stage were significant on most occasions. Brown foot rot disease index was related significantly to the DNA concentration of *M. nivale* at GS 23 and GS 39, but not at GS 83. Take-all incidence correlated significantly with DNA of *Gaeummannomyces graminis* var. *tritici* at GS 83, although the regression accounted for less than 30% of the variance. Eyespot disease index at any growth stage was more consistently associated with DNA concentrations of *O. yallundae* than with DNA of *O. acuformis*. A positive correlation between eyespot symptoms and DNA of *O. acuformis* was observed only at GS 83, when the highest DNA concentrations were detected of more than 6 pg ng^{-1} of total DNA. Symptom expression, although similar for both *Oculimacula* spp., appears related to different pathogen DNA concentrations. In this respect, results from Chapter 3 on the comparable symptom expression of the two *Oculimacula* spp. represent a good example. Results from Chapter 3 indicated that eyespot symptoms caused by *O. acuformis* were less clearly associated with pathogen DNA. Conversely, both visual assessment and pathogen DNA concentration were similarly adequate in predicting severity of eyespot when caused by *O. yallundae*, or pathogen DNA concentration, late in the season. Indeed, in the present study, a significant positive relationship was observed between DNA concentrations of *O. yallundae* at GS 23 and GS 83.

Most of the published literature (Turner *et al.*, 1999; Turner *et al.*, 2001; Turner *et al.*, 2002; Nicholson *et al.*, 2002) on stem-base disease development indicates that mis-identification of symptoms is one of the main limitations in correlating stem-base disease symptoms with the DNA of their pathogens. However, the symptoms of different stem-base diseases are similar during early growth stages of winter wheat and even in mature plants symptoms can occur in close proximity. In addition, there is some evidence suggesting that where stem-base pathogens are present in mixed populations, the symptoms of their respective diseases are often atypical. For example, Polley & Turner (1995) isolated a significant number of isolates of both *Oculimacula* spp. from stems with a range of defined brown foot rot and sharp eyespot symptoms. Bateman (1993) frequently isolated *M. nivale* from stems with eyespot symptoms throughout the growing season. Royle (1998) also reported the absence of typical eyespot symptoms where *O. acuformis* and *M. nivale* were present on wheat stems simultaneously. Turner *et al.* (1999) reported a positive correlation between eyespot incidence and the presence of *M. nivale* var. *majus*, determined by PCR, and also a positive correlation between brown foot rot incidence and both *Oculimacula* spp.

In the first experiment of the present study, eyespot disease index at GS 83 was correlated significantly with DNA concentration of *M. nivale* at GS 23, suggesting that *M. nivale* may have colonised host tissue with early eyespot lesions. Similar observations were made for the second experiment where a significant relationship was observed between eyespot disease index at GS 39 and DNA of *M. nivale* at GS 23. In addition, there was a significant positive relationship between brown foot rot disease index and DNA concentration of *O. yallundae* at GS 39 but not DNA of *O. acuformis*. These results, together with previous studies on the time of symptom expression by *O. yallundae* and *O. acuformis* (Chapter 3), indicate that *O. yallundae* rather than *O. acuformis* is more likely to be involved in the expression of symptoms similar to brown foot rot simply because *O. yallundae* usually produces visible symptoms earlier in the season than *O. acuformis*.

Fusarium spp. were detected in quantifiable amounts on the stem-bases only at GS 83 in the second experiment. Although PCR assays for *Fusarium* spp. were not carried out in the first experiment, the occurrence of significant quantities of these species on stem-bases early in the season is unlikely as other studies have shown that *Fusarium* spp. tend to occur and accumulate much later in the growing season (Royle, 1998; Nicholson *et al.*, 2002) and that *M. nivale* is the predominant pathogen causing brown foot rot in the UK (Parry, 1990). *Gaeumannomyces graminis* var. *tritici*, however, was detected as early as GS 39 in the second experiment and severe infection developed leading to DNA concentration of more than 40 pg ng^{-1} of total DNA of this pathogen in the stem bases by GS 83.

More DNA of *Fusarium* spp. than DNA of *M. nivale* was detected in the grain at GS 92. There was a significant relationship between DNA of *Fusarium* spp. on the stem-bases quantified at GS 83 and DNA found in the grain at GS 92, although the regression accounted for only 13% of the variance. DNA of *M. nivale* quantified in the grain, however, correlated more closely with brown foot rot disease index and *M. nivale* at GS 23 than at GS 83 but again with only 14% of variance accounted for according to regression analysis. These results indicate that stem infection by *M. nivale* or *Fusarium* spp. can be one of the sources of inoculum for ear infection. However, for the subsequent development of ear blight and accumulation of pathogen DNA in the grain, favourable environmental conditions before and after ear infection are likely to play a crucial role. Certainly, the role of rain for the dispersal of inoculum and initiation of ear blight is well documented (Parry *et al.*, 1995).

This is the first report of significant positive relationships between DNA concentrations of *M. nivale* and *Oculimacula* spp. at different growth stages of winter wheat, thus the null hypothesis was rejected. Separate regressions for each growth stage tended to account for a larger proportion of the variance where the DNA concentration of one of the pathogens increased relatively more than the other. For example, in the first

experiment, in which DNA of *Oculimacula* spp. increased more after GS 39 whilst DNA *M. nivale* increased before GS 39, the regressions were significant for all growth stages, fitting parallel lines for both *Oculimacula* spp. and *M. nivale*. In the second experiment, where the DNA of *Oculimacula* spp. increased more prior to GS 39 similarly to DNA of *M. nivale*, a smaller proportion of the variance was accounted for by the separate regressions, and in the case of *O. yallundae*, the data fitted separate lines for each growth stage. The time of greatest DNA increase for *M. nivale* appears consistently to be between GS 23 and GS 39, which may explain why in previous studies on the development of this pathogen the highest DNA concentrations of *M. nivale* have been found following stem extension (Nicholson *et al.*, 2002; Turner *et al.*, 2002). However, increases in DNA of *O. acuformis* and *O. yallundae* have been shown to be more variable throughout the season and possibly dependent on environmental conditions, specifically during the period of stem lesion establishment. The absence of any significant positive relationships between the *Oculimacula* spp. and *M. nivale* between early and late growth stages indicates that there was no predisposition for subsequent development of the species on the host.

Oculimacula yallundae may have a greater competitive ability than *O. acuformis* as suggested by its growth rate and accumulation earlier in the season than *O. acuformis* (Bierman *et al.*, 2002). Conversely, rapid increases in the amounts of *O. acuformis* late in the season after GS 39 have frequently been observed (Chapter 4). However, in this study DNA of *O. yallundae* increased more than DNA of *O. acuformis* between all the growth stages suggesting that competition was more likely to occur between *M. nivale* and *O. acuformis* than between *M. nivale* and *O. yallundae*. The different rates and time of development of the two *Oculimacula* spp. may partially explain why positive associations have been observed less between *O. yallundae* and *M. nivale* and more between *O. acuformis* and *M. nivale*. Bateman & Munnery (1995) considered the possibility that the different rates of development of the *Oculimacula* spp. were based on differences in their ability to tolerate infections by *M. nivale*. The present study, however, failed to provide

clear conclusions in this respect, since increases in DNA of both *Oculimacula* spp. occurred at the same time in each experiment. However, the times of DNA increases of the species were not consistent in both experiments. For example, in the first experiment, where relatively high initial amounts of *M. nivale* were detected, the DNA concentrations of both *Oculimacula* spp. increased later in the growing season, whilst in the second experiment, where similar amounts of all stem-base pathogens were detected, DNA concentrations of both *Oculimacula* spp. increased earlier in the season. Thus, it is possible that the rate and time of DNA increase of the species were influenced by the intitial DNA concentration of *M. nivale*, although different environmental conditions are also likely to affect the subsequent development of the stem-base pathogens in different amounts throughout the growing season and possibly their interactions.

Significant positive relationships were also observed between the DNA of the two *Oculimacula* spp. in both experiments. The DNA data for both species from the three samples over the growing season were fitted best by a common line in the first experiment and parallel lines for each growth stage in the second experiment. These results clearly confirmed existing evidence of the ability of *Oculimacula* spp. to co-exist on stems, indicated earlier by Bateman (1993) and Bierman *et al.* (2002).

It is possible that the different varieties of *M. nivale* interact differently with *Oculimacula* spp. Further research is required to investigate the nature of these relationships.

The severe loss in grain yield, TGW and specific weight in the second experiment was related mainly to take-all incidence and DNA concentration of *G. graminis* var. *tritici* at GS 83, accounting for more than 40 % of the variance. Warm, dry summers (soil temperature of 12-20°C), are known to favour the development of damaging take-all (Hornby *et al.*, 1998). Indeed, the Summer of 2003 was drier and warmer than average, which would have encouraged take-all and led to the high DNA concentrations of *G. graminis* var. *tritici* in the second experiment.

CHAPTER 6

GENERAL DISCUSSION

GENERAL DISCUSSION

Economically eyespot is considered the most important stem-base disease of cereals. Yield losses from the disease are associated with direct effects of severe infection causing reductions in grain yield of up to 30% (Oort, 1936; Glynne & Salt, 1958) and indirect effects of lodging, increasing yield loss by an additional 10% to 15% (Glynne *et al.*, 1945; Jørgensen, 1965; Scott & Hollins, 1974). Furthermore, significant reductions in thousand-grain weight and specific weight are often incurred following severe eyespot epidemics (Deffosse & Rixhon, 1968; Clarkson, 1981).

In the recent past, the causal organisms of eyespot disease were considered as two separate varieties of *Pseudocercosporella herpotrichoides* (Nirenberg, 1981) and their respective isolates were commonly differentiated as W-type and R-type on the basis of host specific pathogenicity (Scott *et al.*, 1975; King & Griffin, 1985), growth rate and morphology in culture (Hollins *et al.*, 1985). Extensive research using biochemical and molecular markers (Julian & Lucas, 1990; Nicholson *et al.*, 1991,1993; Priestley *et al.*, 1992; Thomas *et al.*, 1992; Dyer *et al.*, 1994; Nicholson & Rezanoor, 1994; Robbertse *et al.*, 1995; Crous *et al.*, 2000), however, has demonstrated that W- and R-type isolates are in fact two distinct species, *Oculimacula yallundae* and *O. acuformis*, respectively (Crous *et al.*, 2003).

During the 1980s, significant changes in the population of the eyespot fungi occurred, resulting in the predominance of *O. acuformis* in cereal crops in the UK (King & Griffin, 1985; Birchmore & Russell, 1990; West *et al.*, 1998; Bardsley *et al.*, 1998, Nicholson & Turner, 2000). Most of the past research, concerning the effects of the disease on yield (Scott & Hollins, 1974, 1978; Clarkson, 1981), and the development and use of an economic threshold for eyespot, were based on disease epidemics caused mainly by *O. yallundae*, which was the predominant pathogen in the UK at the time. Following

the shift in the pathogen population in favour of *O. acuformis*, new investigations on the effects on yield and the control of the disease when caused by this pathogen were required in order to re-evaluate the validity of previous work.

Traditionally, visual assessment of eyespot at GS 30/32 (Anon., 1986) was used to determine the need for chemical control of the disease. However, differences in the development of the two *Oculimacula* spp. over the growing season (Goulds & Fitt, 1991a & b) have indicated that using visual assessment early in the crop's growing season for predicting disease at the end of the season was less relevant for eyespot caused by *O. acuformis*. Recently, PCR techniques (Beck *et al.*, 1996; Nicholson *et al.*, 1997) have been employed for the diagnosis and quantification of the pathogens early in the season in an attempt to aid visual assessment and provide more in-depth information on the development of the two species.

Variable results on yield benefit from experiments on the chemical control of eyespot caused by *O. acuformis* (Bateman *et al.*, 2000; Burnett *et al.*, 2000) have suggested that *O. acuformis* was less likely to cause yield loss than *O. yallundae*, possibly because of the tendency of the former species to develop later in the growing season (Royle, 1998). Therefore, further investigation was required on the comparative development of the two *Oculimacula* spp. and the ability of *O. acuformis* to cause yield loss in winter wheat. In addition, any possible factors influencing the differences in species development also required further investigation. One of these possible factors previously suggested by Bateman (1993) and Bateman & Munnery (1995) was differences in the ability of the species to tolerate infections by *Microdochium nivale*, the main causal organism of brown foot rot in the UK. Brown foot rot is often present on the same stem bases as eyespot and, apart from impeding the visual assessment of eyespot early in the season when spray decisions are made, the significance of the frequent co-occurrences of the stem-base pathogens, *M. nivale* and *Oculimacula* spp. on the development of eyespot is unclear.

Results from Chapter 3 and Chapter 4 showed that severe eyespot caused by *O. acuformis* can cause significant yield loss in winter wheat. In agreement with previous reports by Jørgensen (1964) and Scott & Hollins (1974), the measured losses in ear weight were primarily associated with severe lesions of eyespot caused by both *Oculimacula* spp. and overall yield in the absence of lodging was reduced by 11% and 6% by eyespot caused by *O. acuformis* and *O. yallundae*, respectively. Thus, previous studies on the effects of eyespot on yield of winter wheat appeared valid for the disease caused by either pathogen. Results from Chapter 3 also indicated that the effects of the disease on plant characteristics associated with lodging were similar for both species but dependent on different pathogen DNA concentrations (presumably indicating different biomass for each pathogen). Reductions in the stem safety factor, associated with lodging resistance of winter wheat, were principally explained by relative reductions in stem bending strength in the diseased plants. In comparison, reductions in the stem safety factor of disease-free plants are mostly related to reductions in the self-weight moment (height and weight of plant shoots) of the plants. The observed differences among isolates of *O. acuformis* and *O. yallundae* in their effects on plant characteristics associated with lodging indicated that the intrinsic aggressiveness of individual isolates within species plays an important role in the development and effects of eyespot epidemics.

Differences between the two *Oculimacula* spp. in disease incidence and severity early and late in the growing season (Chapter 3) were consistent with previous reports (Goulds & Fitt, 1988; Bateman *et al.*, 1990; Goulds & Fitt, 1991a & b). Incidence and severity of eyespot symptoms at an early crop growth stage (GS 33) were greater when the disease was caused by *O. yallundae*. However, these differences between species were not apparent late in the season (after GS 60).

Visual assessments of eyespot caused by *O. yallundae* consistently correlated with its pathogen DNA concentrations at each growth stage, and either visual or PCR data from GS 33 successfully predicted disease severity and pathogen abundance at the end of the

growing season. However, as shown in Chapter 5, where brown foot rot was present on the same stem bases as eyespot, pathogen DNA concentration, rather than visual disease assessment, at the early growth stages was more reliable in predicting amounts of *O. yallundae* at the end of the season. These strong relationships between pathogen DNA and severity of eyespot caused by *O. yallundae* between growth stages (Chapters 3 & 5) were associated with consistent relationship between symptom expression by this pathogen and increases in its DNA throughout the season. This clearly suggests that the use of the traditional economic threshold is still appropriate and relevant to situations where the disease is caused predominantly by *O. yallundae*. In comparison, symptom expression by *O. acuformis* was less dependent on pathogen DNA, as indicated by the lack of significant relationships between visual assessment and amounts of DNA of *O. acuformis* detected at various growth stages. In addition, the noticeable absence of significant relationship between visually assessed eyespot caused by *O. acuformis* early and late in the season (Chapters 3, 4 & 5) clearly indicated that the use of the traditional threshold for eyespot where the dominant pathogen is *O. acuformis* would be highly ineffective in assessing the need for disease control. Furthermore, visual assessment was confounded on most occasions by the presence of brown foot rot (Chapters 4 & 5). Thus, PCR assays were clearly more valuable in providing positive identification and quantification of specific pathogens, particularly as a guide for making decisions on fungicide use. In this respect, pathogen DNA quantified at the early crop growth stages was a more reliable indicator of disease and concentrations of *O. acuformis* later in the season. Relationships were stronger, however, when DNA quantification was made as close as possible to GS 39 (Chapters 3 & 4) rather than at GS 30/32, when fungicides are actually applied. This is not unexpected, since it coincides with the period of stem lesion establishment, essential for the subsequent development of the disease in adult plants (Goulds & Fitt, 1991a & b), which in turn may explain why rapid increases in the DNA of *O. acuformis* have been observed more often following stem extension of cereals.

Results from Chapter 4 on the effects of a range of fungicides on eyespot caused by *O. acuformis* in early-drilled first, second and third winter wheats showed that the application of fungicide mixtures that included cyprodinil resulted most consistently in effective eyespot control, and significant yield increases, particularly in situations where *O. acuformis* was dominating the stem-base pathogen population early in the season.

Rapid increases in DNA of *O. acuformis* resulting in more than 30 pg ng^{-1} of total DNA at the end of the season, associated with significant yield loss, occurred only in the inoculated experiment (Chapter 3) and in a first winter wheat of Consort (Chapter 4). Thus, greater yield loss from eyespot caused by *O. acuformis* is most likely to occur where pathogen DNA concentration late in the season reaches more than 10 pg ng^{-1} of total DNA. Indeed, *O. acuformis* has clearly shown the ability to increase its DNA by more than 10-fold between sampling times in all experiments (Chapters 3, 4 & 5). Nevertheless, concentrations of *O. acuformis* failed to exceed 10 pg ng^{-1} of total DNA at the end of the season in the rest of the experiments described here. The experiments in which *O. acuformis* reached high DNA concentrations late in season and high yield loss was observed, appeared to have one common factor. This was the absence, or the relatively low occurrence of other stem-base pathogens early in the season, up to GS 39. These observations provided additional information on the effects of stem-base pathogens on the development of eyespot (Chapter 5).

Results presented in Chapter 5 showed that DNA of *O. acuformis*, *O. yallundae* and *M. nivale* increased throughout the season and positive relationships between pathogen DNA of the three species were present for each growth stage, indicating that the species were able to co-exist and grow in the same crop. However, there were no significant relationships for amounts of DNA of the stem-base pathogens between early and late growth stages. In addition, both *Oculimacula* spp. showed similar patterns of development, which however, were not consistent between the two experiments. Where relatively high levels of *M. nivale* were present at early growth stages (GS 20/39) of the

crop, *Oculimacula* spp. increased later in the growing season; where when initial differences between stem-base pathogens were less noticeable *Oculimacula* spp. increased earlier in the season. DNA of *M. nivale* consistently increased more up to GS 39 (Chapters 4 & 5), indicating that, this is the main period of development and DNA accumulation for this pathogen. DNA of *Oculimacula* spp., nevertheless, increased at different times that often appeared related to the initial *M. nivale* concentrations. These differences may indicate a requirement for different environmental conditions that may have affected patterns of development. However, since the two experiments in Chapter 5 were carried out in the same year and in close proximity it is unlikely that the observed differences in eyespot development were caused by major differences in environmental conditions. Another possible explanation is differences in the agronomy of the crops. Although, these were minimised where possible, rates and forms of fertiliser and fungicides applied for the control of other foliar diseases may have had some effect.

Although the sequence of infection by the stem-base pathogens is unclear, it is likely that the pathogen with most competitive advantage would be the one that infects earlier than the others and has a faster growth rate. In this respect, because *O. acuformis* lacks the ability to grow as fast as *O. yallundae* or *M. nivale*, it may have a greater competitive advantage in crops where it infects earlier than other stem-base pathogens which are absent or in relatively small amounts. This may explain the somewhat high DNA concentrations of *O. acuformis* detected at the end of the season in the first winter wheat of Consort (Chapter 4). In conjunction with the indications that more than 10 pg ng^{-1} of *O. acuformis* of total DNA are required to induce significant yield loss, it may also suggest that early drilled winter wheat crops in which lower initial populations of other stem-base pathogens are present up to GS 39 are potentially at greater risk of yield loss from severe eyespot caused by *O. acuformis*. In this respect, winter wheat crops following a one- or two-year break from cereals, particularly where ploughing has been practised, so

that crop debris infested with other stem-base pathogens has been buried, will most likely fit the category of high risk crop.

There was more evidence for competition between *M. nivale* and *O. acuformis* than between *M. nivale* and *O. yallundae*, since *O. acuformis* failed to increase its DNA as much as *O. yallundae* in the presence of *M. nivale*.

Main conclusions

- *Oculimacula acuformis* is capable of causing significant yield loss in winter wheat. Both *Oculimacula* spp. can reduce the stem safety factor associated with lodging resistance by reducing the stem bending strength.
- The effects on lodging and plant characteristics associated with it may also be influenced by the aggressiveness of individual isolates within each *Oculimacula* spp.
- Differences in pathogen development and symptom expression by the two *Oculimacula* spp. have indicated that the current threshold is more valid for disease caused by *O. yallundae* than *O. acuformis*.
- Prediction of disease or pathogen DNA late in the season using visual assessment was consistent only for disease caused by *O. yallundae*. Prediction of abundance of *O. acuformis* late in the season was more reliable using pathogen DNA at an early growth stage (GS 33) than visual assessment at the same growth stage.
- Effective eyespot control resulting in significant yield increases can be achieved by using fungicide mixtures inclusive of cyprodinil, particularly in situations where *O. acuformis* is causing the disease and is dominating the stem-base pathogen populations early in the season.

- Different stem-base pathogens develop at different rates, which may vary depending on different crops or environmental conditions. However, greatest increases in DNA of *M. nivale* have occurred most consistently up to GS 39. DNA of *Oculimacula* spp., nevertheless, increased at different times that often appeared related to the initial *M. nivale* concentrations.
- The positive correlations between DNA of stem-base pathogens present throughout the growing season indicated that *M. nivale*, *O. acuformis* and *O. yallundae* probably co-exist and are generally not antagonistic. Nevertheless, a stem-base pathogen prevalent early in the season has some competitive advantage and remains prevalent later in the season.
- Competition is more likely to occur between *M. nivale* and *O. acuformis* than *M. nivale* and *O. yallundae*, whilst *O. acuformis* and *O. yallundae* generally do not interact negatively.
- Initial stem-base pathogen populations and concentrations may be more important for the subsequent development of eyespot caused by *O. acuformis* than by *O. yallundae*.

FURTHER RESEARCH

- Results from Chapter 3 indicated that effects of the two *Oculimacula* spp. on plant characteristics and lodging are influenced by the intrinsic aggressiveness of individual isolates within the species. Therefore, further investigation on the effects of yield and associated plant properties is required using single isolates of *Oculimacula* spp. in field conditions.
- Results from Chapters 3 & 4 indicated that eyespot caused by *O. acuformis* may cause greater yield loss in first winter wheat crops, where smaller amounts of other stem-base pathogens are usually present early in the season. Results from Chapter 5 also indicated that DNA concentration of *M. nivale* early in the season may be relevant to risk assessment of yield loss caused by eyespot and the development of a threshold for *O. acuformis*. In this respect, a survey that includes early and late sampling of first winter wheat crops and DNA quantification for *O. acuformis*, *O. yallundae* and *M. nivale*, in addition to crop yields, determined on single-shoot basis or whole crop may help in the development of a new threshold for *O. acuformis*
- As indicated by the DNA quantification data for *O. acuformis* in Chapters 3 & 4, it may be useful to re-evaluate timing of fungicide application. Delaying applications as close as possible to GS 37/39 without losing fungicide efficacy may be more appropriate for eyespot caused by *O. acuformis* and it may possibly be used in conjunction with a meaningful threshold based on pathogen DNA concentration at this growth stage.
- For eyespot caused by *O. acuformis* and possible threshold development, further examination of the size of early sampling (number of plants/main shoots) and/or, amount of plant material used for DNA extraction may be worthwhile. Pathogen DNA present on the outer leaf sheaths (which senesce and die during plant growth) and on

the living shoot, without the outer leaf sheaths may be examined separately to determine their relative contribution to later pathogen accumulation on the stems of adult plants.

- The sequence of infection by *M. nivale* and *Oculimacula* spp. and the different environmental conditions favourable for the diseases can be further examined in controlled environment studies, using different inoculation times and conidial inoculum of *M. nivale* and *Oculimacula* spp. at different concentrations in addition to seed infected with *M. nivale*.
- A series of field experiments with different amounts of artificial oat-grain inoculum of *M. nivale* and *Oculimacula* spp., applied at the same time on winter wheat with three or more sampling times may determine:
    1. Rates and periods of development of the two *Oculimacula* spp. in relation to different initial pathogen populations, particularly of *M. nivale,* in order to establish the relative importance of such initial pathogen populations and amounts, for eyespot development and consequent effect on yield on individual stems and whole crop.
    2. Interactions between the two *Oculimacula* spp. and the two *M. nivale* varieties, *majus* and *nivale*, which may also be important in relation to changes in the stem-base pathogen population over the growing season.

BIBLIOGRAPHY

Adam, D.B. (1940). On the occurrence of *Cercosporella herpotrichoides* Fron in South Australia. *The Journal of Australian Institute of Agricultural Science* **6,** 48-51.

Albertini, C., Gredt, M., Leroux, P. (1999). Mutations of the β - tubulin gene associated with different phenotypes of benzimidazole resistance in the cereal eyespot fungi *Tapesia yallundae* and *Tapesia acuformis*. *Pesticide Biochemistry and Physiology* **64**, 17-31.

Albertini, C., Gredt, M., Leroux, P. (2003). Polymorphism of 14 alpha-demethylase gene (*CYP51*) in the cereal eyespot fungi *Tapesia acuformis* and *Tapesia yallundae*. *European Journal of Plant Pathology* **109**, 117-128.

Anon. (1961). Distribution maps of plant diseases. *Cercosporella herpotrichoides*. Map No.74, 4th edition. Commonwealth Mycological Institute.

Anon. (1986). Winter wheat - managed disease control. MAFF, ADAS, Advisory leaflet 831, Alnwick 1986.

Anon. (1992). Beckmann Instruments, Nucleic acid analysis. Manual No. 517304B. Beckmann Inc., USA.

Anon. (2002). Pocket Guide to Varieties of Cereals and Oilseeds & Pulses. NIAB, Cambridge.

Arx, J. A. von. (1983). *Mycosphaerella* and its anamorphs. *Biol. Med. Science* C **86**, 15-54.

Babij, J., Zhu, Q., Brain, P., Hollomon, D.W. (2000). Resistance risk assessment of cereal eyespot, *Tapesia yallundae* and *Tapesia acuformis*, to the anilinopyrimidine fungicide, cyprodinil. *European Journal of Plant Pathology* **106**, 895-905.

Bardsley, E.S., Burgess, J., Daniels, A., Nicholson, P. (1998). The use of polymerase chain reaction diagnostic test to detect and estimate the severity of stem base diseases in winter wheat. In: Proceedings of the Brighton Crop Protection Conference. British Crop Protection Council, Farnham, UK, pp. 1041-1046.

Bateman, G.L., Edwards, S.G., Marshall, J., Morgan, L.W., Nicholson, P., Nuttall, M., Parry, D.W., Scrancher, M., Turner, A.S. (2000a). Effects of cultivar and fungicides on stem-base pathogens, determined by quantitative PCR, and on diseases and yield of wheat. *Annals of Applied Biology* **137**, 213-221.

Bateman, G. L., Edwards, S.G., Marshall, J., Morgan, L.W., Nicholson, P., Nuttall, M., Parry, D.W., Scrancher, M., Turner, A.S. (2000b). Diagnosis, forecasting, risk assessment and control of stem-base diseases of wheat using new molecular technologies. Project Report No.216. Home Grown Cereals Authority, London, pp. 145.

Bateman, G. L., Jenkyn, J.F. (2000). Towards better understanding and management of cereal stem - base diseases. Proceedings of the BCPC Conference - Pests and Diseases 2000, 119-126.

Bateman, G. L., Landau, S., Welham, S.J. (1995). Sensitivity to prochloraz in populations of the eyespot fungus, *Pseudocercosporella herpotrichoides*, in relation to fungicide treatments and their efficacy in continous winter wheat. *Annals of Applied Biology* **126,** 235-247.

Bateman, G.L. (1990). Comparison of the effects of prochloraz and flusilazole on foot rot diseases and on populations of the eyespot fungus, *Pseudocercosporella herpotrichoides*, in winter wheat. *Zeitschrift für Pflanzenkrankheiten und Planzenschutz* **97**, 508-516.

Bateman, G.L. (1993). Development of disease symptoms and fungal pathogens on shoot bases in continuous winter wheat, and effects of fungicides. *Plant Pathology* **42**, 595-608.

Bateman, G.L. (2002). Long-term performance of fungicides applied to control eyespot in winter wheat. *Annals of Applied Biology* **141**, 29-33.

Bateman, G.L., Fitt, B.D.L. (1991). Effects of fungicides on eyespot in five successive crops of winter wheat in plots with different initial fungicide-selected populations *of Pseudocercosporella herpotrichoides*. *Crop Protection* **10,** 479-484.

Bateman, G.L., Fitt, B.D.L., Creighton, N.F., Hollomon, D.W. (1990b). Changes in populations of *Pseudocercosporella herpotrichoides* in successive crops of winter wheat in relation to initial populations and fungicide treatments. *Crop Protection* **9**, 135-142.

Bateman, G.L., Goulds, A., Ainsley, A. (1990a). Comparison of infection by W-type and R-type isolates of the eyespot pathogen, *Pseudocercosporella herpotrichoides*, in relation to host species and cultivar, temperature and watering regime in controlled environments. *Journal of Phytopathology* **129,** 271-282.

Bateman, G.L., Gutteridge, R.J. (1996). Incidence of eyespot and selection of W-type and R-type of *Pseudocercosporella herpotrichoides* in sequences of different cereal species. *Aspects of Applied Biology* **47**, 437-440.

Bateman, G.L., Jenkyn, J.F. (2001). Biology and control of stem-base diseases of cereals. *Pesticide Outlook – June 2001*, 103-106.

Bateman, G.L., Munnery, I.C. (1995). Development of eyespot and brown foot rot in wheat plants inoculated with mixtures of *Pseudocercosporella* types and *Fusarium* species. *Journal of Phytopathology* **143**, 147-151.

Bateman, G.L., Taylor, G.S. (1976a). Significance of the coleoptile in establishment of seedling infection on wheat by *Pseudocercosporella herpotrichoides*. *Transactions of the British Mycological Society* **67**, 513-514.

Bateman, G.L., Taylor, G.S. (1976b). Seedling infection of two wheat cultivars by *Pseudocercosporella herpotrichoides*. *Transactions of the British Mycological Society* **67**, 95-101.

Beck, J. (1997). Development of PCR assays to detect *Fusarium* spp. in wheat. (abstr) APS Meeting, Aug. 9-13. Rochester, New York. *Phytopathology* **87**, S7

Beck, J., Beebe, J., Stewart, S., Bassin, C., Etienne, L. (1996). Colorimetric PCR and ELISA diagnostics for the detection of *Pseudocercosporella herpotrichoides* in field samples. In: Proceedings of the Brighton Crop Protection Conference. British Crop Protection Council, Farnham, UK, pp. 221-226.

Beck, J.J., Beebe, J.R., Stewart, S.J., Bassin, C., Etienne, L. (1996). Colorimetric PCR and ELISA diagnostics for the detection of *Pseudocercosporella herpotrichoides* in field samples. In: Proceedings of the Brighton Crop Protection Conference. British Crop Protection Council, Farnham, UK, pp. 221-226.

Bierman, S.M., Fitt, B.D.L., Bosh, F. van den, Bateman, G.L., Jenkyn, J.F., Welham, S.J. (2002). Changes in the populations of the eyespot fungi *Tapesia yallundae* and *T. acuformis* under different fungicide regimes in successive crops of winter wheat, 1984-2000. *Plant Pathology* **51**, 191-201.

Birchmore, R.J., Russel, P.E. (1990). Long-term monitoring of *Pseudocercosporella herpotrichoides* populations for sensitivity to prochloraz and carbendazim. In: Proceedings of the Brighton Crop Protection Conference. British Crop Protection Council, Farnham, UK, pp. 1153-1158.

Birchmore, R.J., Vernie, P., Russel, P.E., Buschhaus, H. (1986). Sensitivity of *Pseudocercosporella herpotrichoides* populations to prochloraz. In: Proceedings of the Brighton Crop Protection Conference, British Crop Protection Council. Farnham, UK, pp.787-792.

Blair, I.D. (1954). Nutrition and survival of *Cercosporella herpotrichoides* Fron and aspects of wheat eyespot disease. *New .Zealand Jounal of Science and Technology* **36**, 207-220.

Boerema, G.H., Pieters, R., Hamers, M.E.C. (1992). Check-list for scientific names of common parasitic fungi. Supplement Series 2b (additions and corrections): Fungi on field crops: cereals and grasses. *Netherlands Journal of Plant Pathology* **98**, 1-32.

Booth, C.and Waller, J.M. (1973). *Pseudocercosporella herpotrichoides*. CMI Descriptions of Pathogenic Fungi and Bacteria No.388.

Brown, M.C., Taylor, G.S., Epton, H.A.S. (1984). Carbendazim resistance in the eyespot pathogen *Pseudocercosporella herpotrichoides*. *Plant Pathology* **33,** 101-111.

Brück, K.P. and Schlösser, E. (1982). Cereal foot rot pathogens. V. Antagonism between pathogens. *Journal of Plant Diseases and Protection* **89**, 337-343.

Bruehl, G.W. and Nelson, W.L. (1964). Technique for mass inoculations of winter wheat in the field with *Cercosporella herpotrichoides*. *Plant Disease Reporter* **48**, 863-865.

Bruehl, G.W., Lai, P. (1966). Prior-colonisation as a factor in the saprophytic survival of several fungi in wheat straw. *Phytopathology* **56**, 766-768.

Bruehl, G.W., Manandhar, J.B. (1972). Some water relations of *Cercosporella herpotrichoides*. *Plant Disease Reporter* **56**, 594-596.

Bruehl, G.W., Nelson, W.L., Kowhler, F., Vogel, O.A. (1968). Experiments with *Cercosporella* foot rot (straw breaker) disease of winter wheat. Bulletin of the Washington Agricultural Experiment Station 694, 1-14.

Burnett, F. J. (1999). The use of fungicide sequences to maximise the control of eyespot in cereals and minimise the risk of sharp eyespot. Project Report No. 200. Home Grown Cereals Authority, London, pp. 3-32.

Burnett, F.J., Oxley, S.J.P., Harling, R. (1997). The use of PCR diagnostics to monitor development of eyespot in winter wheat. HGCA Project report No.150. Home Grown Cereals Authority, London, pp. 25.

Burnett, F.J., Oxley, S.J.P., Laing, A.P. (2000). The use of PCR diagnostics in determining eyespot control strategies in wheat. In: Proceedings of the Brighton Crop Protection Conference. British Crop Protection Council, Farnham, UK, pp. 107-112.

Bush, N., Bardsley, E.S. (1988). Control of stem base and foliar diseases of winter cereals with prochloraz plus cyproconazole. In: Proceedings of the Brighton Crop Protection Conference. British Crop Protection Council, Farnham, UK, pp.935-940.

Butler, F.C. (1957). Eyespot lodging of wheat: A new record for New South Wales. *Agricultural Gazette of New South Wales* **68,** 340-343.

Byther, R. S., Powelson, R.L. (1966). Observations on *Cercosporella herpotrichoides* in soil. *Phytopathology* **56**, 1314-1315.

Cavelier, N. (1994). Pourra-t-on jamais en finir avec le piétin-verse? Première observation en France de la frome sexuée du champignon. *Phytoma* **462**, 17.

Cavelier, N. (1998). Variability of the sensitivity of different isolates of *Pseudocercosporella herpotrichoides* to sterol biosynthesis inhibitor fungicides. Cereal Breeding related to the Integrated Cereal Production. In: Proceedings of the Conference of the Cereal Section of Eucarpia, Wageningen, The Netherlands, pp. 140-144.

Cavelier, N., Rousseau, M., Le Page, D. (1987). Variability of *Pseudocercosporella herpotrichoides*, the cause of the eyespot disease: in vivo behaviour of two types isolates and a mixed population. *Journal of Plant Diseases and Protection* **94**, 590-599.

Chang, E.P., Tyler, L.J. (1964). Sporulation by *Cercosporella herpotrichoides* on artificial media. *Phytopathology* **54,** 729-735.

Clarkson, J.D.S. (1981). Relationship between eyespot severity and yield loss in winter wheat. *Plant Pathology* **30**, 125-131.

Clarkson, J.P., Lucas, J.A. (1993). Screening for potential antagonists of *Pseudocercosporella herpotrichoides*, the causal agent of eyespot disease of cereals. 2. Bacteria. *Plant Pathology* **42**, 543-551.

Clarkson, J.P., Lucas, J.A. (1997). The role of antibiotic production by a strain of *Pseudomonas fluorescens* in the suppression of *Pseudocercosporella herpotrichoides*, the causal agent of eyespot disease of cereals. *Journal of Applied Microbiology* **82**, 499-506.

Coff, C., Poupard, P., Xiao, Q., Graner, A., Lind, V. (1998). Sequence of plastocyanin cDNA from wheat and the use of the gene product to determine serologically tissue degradation after infection by *Pseudocercosporella herpotrichoides*. *Journal of Phytopathology* **146**, 11-17.

Colbach, N., Duby, C., Cavelier, A., Meynard, J.M. (1997). Influence of cropping systems on foot and root diseases of winter wheat: fitting of a statistical model. *European Journal of Agronomy* **6**, 61-77.

Colbach, N., Maynard, J.M. (1995). Soil tillage and eyespot: influence of crop residue distribution on disease development and infection cycles. *European Journal of Plant Pathology* **101**, 601-611.

Colbach, N., Maynard, J.M., Duby, C., Huet, P. (1999). A dynamic model of the influence of rotation and crop management on the disease development of eyespot. Proposal of cropping systems with low disease risk. *Crop Protection* **18**, 451-461.

Colbach, N., Saur, L. (1998). Influence of crop management on eyespot development and infection cycles of winter wheat. *European Journal of Plant Pathology* **104**, 37-48.

Cook, R.J., Polley, R.W., Thomas, M.R. (1991). Disease-induced losses in winter wheat in England and Wales 1985-1989. *Crop Protection* **10**, 504-508.

Cooke, B.K., Hislop, E.C., Jordan, V.W., Western, N.M., Herrington, P.J. (1989). Redistribution of foliar surface deposits of prochloraz by simulated rainfall and the control of eyespot disease of winter wheat. *Crop Protection* **8**, 373-379.

Cooke, B.K., Jordan, V.W.L., Hislop, E.C., Western, N.M. (1993). Mechanism of control of eyespot disease in winter wheat by the fungicide prochloraz. *Crop Protection* **12**, 279-283.

Coşkun, H., Bateman, G.L., Hollomon, D.W. (1987). Changes in population structure of carbendazim - resistant eyespot in wheat and barley between spring and summer 1984. *Transactions of the British Mycological Society* **88**, 117-119.

Covarelli, L., Santori, A. (2000). Influenza dell'epoca di semina e di trattamenti fungicide sul mal del piede e sulla septoriosi del frumento tenero (*Triticum aestivum L.*) in condizioni di monosuccessione prolungata. Risultati preliminary. ATTI *Giornale Fitopatologiche* **2**, 253-258.

Cox, J., Cock, L. J. (1962). Survival of *Cercosporella herpotrichoides* on naturally infected straws of wheat and barley. *Plant Pathology* **11**, 65-66.

Creighton, N.F. (1989). Identification of W-type and R-type isolates of *Pseudocercosporella herpotrichoides*. *Plant Pathology* **38**, 484-493.

Creighton, N.F., Bateman, G.L. (1990). Problems in identifying pathogenicity types *of Pseudocercosporella herpotrichoides* by colony morphology arising from the source and concentration of the nutrient agar. *Mycological Research* **95**, 253-254.

Creighton, N.F., Cavelier, N., Fitt, B.D.L. (1989). Pathogenicity to wheat and rye of *Pseudocercosporella herpotrichoides* isolates from France and UK. *Mycological Research* **92**, 13-17.

Crook, M., Ennos, A.R. (1994). Stem and root characteristics associated with lodging resistance in four winter wheat cultivars. *Journal of Agricultural Science, Cambridge* **123**, 167-174.

Crook, M., Ennos, A.R. (2000). A field based method of quantifying the lodging resistance of wheat cultivars. In: Plant Biomechanics 2000, Proceedings of the 3rd Plant Biomechanics Conference Freiburg-Badenweiler, 315-320.

Crous, P.W., Aptroot, A., Kang, J-Ch, Braun, U., Wingfield, M. J. (2000). The genus *Mycosphaerella* and its anamorphs. *Studies in Mycology* **45**, 107-121.

Crous, P.W., Groenewald, J.Z.E., Gams, W. (2003). Eyespot of cereals revisited: ITS phylogeny reveals new species relationships. *European Journal of Plant Pathology* **109**, 841-850.

Cunningham, P. C. (1966). Influence of nitrogen on the incidence of foot and root rots of spring-sown cereals. *Irish Journal of Agricultural Research* **5**, 63-78.

Cunningham, P.C. (1981). Occurrence, role and pathogenic traits of a distinct pathotype of *Pseudocercosporella herpotrichoides*. *Transactions of the British Mycological Society* **76**, 3-15.

Daniels, A., Lucas, J.A. (1990). Influence of prochloraz on the early pathogenesis of *Pseudocercosporella herpotrichoides*. In: Proceedings of the Brighton Crop Protection Conference. British Crop Protection Council, Farnham, UK, pp. 847-854.

Daniels, A., Lucas, J.A., Peberdy, J.F. (1991). Morphology and ultrastructure of W and R pathotypes of *Pseudocercosporella herpotrichoides* on wheat seedlings. *Mycological Research* **95**, 385-397.

Daniels, A., Papaikonomou, M., Dyer, P.S., Lucas, J.A. (1995). Infection of wheat seedlings by ascospores of *Tapesia yallundae*: morphology of the infection process and evidence for recombination. *Phytopathology* **85**, 918-927.

de La Peña, R.C., Murray, T.D. (1994). Identifying wheat genotypes resistant to eyespot disease with b - glucuronidase - transformed strain of *Pseudocercosporella herpotrichoides*. *Phytopathology* **84**, 972-977.

de La Peña, R.C., Murray, T.D., Jones, S.S. (1996). Linkage relations among eyespot resistance gene *Pch2*, endopeptidase *Ep-A1b*, and RFLP marker *Xpsr121* on chromosome 7A of wheat. *Plant Breeding* **115**, 273-275.

de La Peña, R.C., Murray, T.D., Jones, S.S. (1997). Identification of an RFLP interval containing *Pch2* on chromosome 7AL in wheat. *Genome* **40**, 249-252.

Deacon, J.W. (1973a). Behaviour of *Cercosporella herpotrichoides* and *Ophiobolus graminis* on buried wheat plant tissues. *Soil Biology and Biochemistry*. **5**, 339-353.

Deacon, J.W. (1973b). Pseudoparenchyma produced by *Cercosporella herpotrichoides* in culture. *Transactions of the Brithish Mycological Society* **60**, 537-545.

Defosse, L. (1966). Les premiers stages de l'infection du *Cercosporella herpotrichoides* Fron sur froment, orge, seigle et avoine. Bulletin des Recherches Agronomiques de etude en conditions experimentales Gembloux 1, 561-569.

Defosse, L., Rixhon, L. (1968). Influence d'une serie de precedents culturaux sur les pietins du froment (*Cercosporella herpotrichoides* Fron et *Ophiobolus graminis* Sacc.). *Parasitica* **24**, 107-120.

Deighton, F.C. (1973). Studies on *Cercosporella* and allied genera. IV. *Cercosporella* Sacc., *Pseudocercosporella* gen.nov. and *Pseudocercosporidium* gen.nov. *Mycological Papers* **133**, 46.

Dennis, R.W.G. (1944). Cereal diseases in Scotland. *Annals of Applied Biology* **31**, 370-374.

Douhan, G. W., Murray, T.D., Dyer, P.S. (2002). Species and mating-type distribution of *Tapesia yallundae* and *T. acuformis* and occurrence of apothecia in the US Pacific Northwest. *Phytopathology* **92**, 703-709.

Doussinault, G. (1970). Problemes poses par l'amelioration de la resistance du ble tendre vis-à-vis du pietin-verse *Cercosporella herpotrichoides* Fron. *Annales de l'amélioration des plantes* **20**, 433-452.

Doussinault, G., Dosba, F, Jahier, J. (1983). New results on the improvement of the level of resistance to eyespot in wheat. Proceedings of the 6th International Wheat Genetics Symposium, Kyoto, Japan, 1983, 193-198.

Drath, M.J., Rapilly, F. (1967). Sporulation en culture pure et a partir de stromas du *Cercosporella herpotrichoides* Fron. *Annalles Epiphyties* **18**, 85-91.

Dyer, P.S., Bateman, G.L., Lucas, J.A., Peberdy, J.F. (1994). Seasonal development of apothecia of the cereal eyespot pathogen *Tapesia yallundae* on straw stubble in the UK. *Annals of Applied Biology* **125**, 489-500.

Dyer, P.S., Bradshaw, R.E. (2002). First report of apothecia of *Tapesia yallundae* occurring on the wild grass *Holcus lanatus* (Yorkshire Fog) in New Zealand. *Plant Pathology* **51**, 806.

Dyer, P.S., Furneaux, P.A., Douhan, G., Murray, T.D. (2001). A multiplex PCR test for determination of mating type applied to the plant pathogens *Tapesia yallundae* and *Tapesia acuformis*. *Fungal Genetics and Biology* **33**, 173-180.

Dyer, P.S., Hansen, J., Delaney, A., Lucas, J.A. (2000). Genetic control of resistance to the sterol 14α-Demethylase inhibitor fungicide prochloraz in the cereal eyespot pathogen *Tapesia yallundae*. *Applied and Environmental Microbiology* **66**, 4599-4604.

Dyer, P.S., Lucas, J.A. (1995). Incidence of apothecia of *Tapesia yallundae* at set-aside sites in England and sensitivity of the ascospores offspring to the fungicides benomyl and prochloraz. *Plant Pathology* **44**, 796-804.

Dyer, P.S., Nicholson, P., Rezanoor, H.N., Lucas, J.A., Peberdy, J.F. (1993). Two-allele heterothallism in *Tapesia yallundae*, the teleomorth of the cereal eyespot pathogen *Pseudocercosporella herpotrichoides*. *Physiological and Molecular Plant Pathology* **43**, 403-414.

Dyer, P.S., Nicholson, P., Rezanoor, H.N., Lucas, J.A., Peberdy, J.F. (1996). *Tapesia acuformis* as a causal agent of eyespot disease of cereals and evidence for a hereothallic mating system using molecular markers. *Mycological Research* **100**, 1219-1226.

Dyer, T.A., Bowman, C.M. (1987). Causes and uses of structural variation in the chloroplast genomes of *Triticum* and *Aegilops* species. Annual Report of the Plant Breeding Institute 1986, pp. 80-82.

Edwards, S.G., Pirgozliev, S.R., Hare, M.C., Jenkinson, P. (2001). Quantification of trichothecene-producing *Fusarium* species in harvested grain by competitive PCR to determine efficacies of fungicides against *Fusarium* head blight of winter wheat. *Applied and Environmental Microbiology* **67**, 1575-1580.

Evans, C.A.B., Davies, W.P. (1986). Fungicide and cultivar interactions with eyespot infections of wheat. *Tests of Agrochemicals and Cultivars, 7 - A Supplement to Annals of Applied Biology* **108**, 78-79.

Fatemi, F., Fitt, B.D.L. (1983). Dispersal of *Pseudocercosporella herpotrichoides* and *Pyrenopeziza brassicae* spores in splash droplets. *Plant Pathology* **32**, 401-404.

Fehrmann, H, Horsten, J., Siebrasse, G. (1982). Five years' results from a long-term field experiment on carbendazim resistance of *Pseudocercosporella herpotrichoides* (Fron) Deighton. *Crop Protection* **1**, 165-168.

Fehrmann, H. & Schrödter, H. (1973). Control of *Cercosporella herpotrichoides* in winter wheat in Germany. In: Proceedings of the 7th British Insecticide and Fungicide Conference. British Crop Protection Council, Farnham, UK, pp. 119-126.

Fitt, B. D.L. (1985). Factors affecting the development of eyespot (*Pseudocercosporella herpotrichoides*) lesions in wheat. *Journal of Plant Diseases and Protection* **95**, 455-463.

Fitt, B. D.L. (1988). Eyespot disease of cereals. Research Review No. 1. Home Grown Cereals Authority, London, pp. 7-24.

Fitt, B.D.L., Bainbridge, A. (1983). Dispersal of *Pseudocercosporella herpotrichoides* spores from infected wheat straw. *Journal of Phytopathology* **106**, 214-225.

Fitt, B.D.L., Creighton, N.F., Bateman, G.L. (1987). Pathogenicity to wheat seedlings of wheat-type and rye-type isolates of *Pseudocercosporella herpotrichoides*. *Transactions of the British Mycological Society* **88**, 149-155.

Fitt, B.D.L., Goulds, A., Polley, R.W. (1988). Eyespot (*Pseudocercosporella herpotrichoides*) epidemiology in relation to prediction of disease severity and yield loss - a review. *Plant Pathology* **37**, 311-328.

Fitt, B.D.L., Nijman, D.J. (1983). Quantitative studies on dispersal of *Pseudocercosporella herpotrichoides* spores from infected wheat straw by simulated rain. *Netherlands Journal of Plant Pathology* **89**, 198-202.

Fitt, B.D.L., White, R.P. (1988). Stages in the progress of eyespot epidemics in winter wheat crops. *Journal of Plant Diseases and Protection* **95**, 35-45.

Foëx, E., Rosella, E. (1933). Quelques observations sur le Pietin des cereales. Review. *Patl. Veg. Et d'Ent.Agr.* **20**, 172-187.

Förster, E., (1994). An improved general method to generate internal standards for competitive PCR. *BioTechniques* **16**, 18-20.

Frei, P., Gindrat, D. (1995). Diversity in culture morphology, sensitivity to carbendazime and the pathogenisis of *Tapesia yallundae* (anamorph: *Pseudocercosporella herpotrichoides*). *Canadian Journal of Botany* **73**, 1379-1384.

Frei, U., Wenzel, G. (1993). Differentiation and diagnosis of *Pseudocercosporella herpotrichoides* (Fron) Deighton with genomic DNA probes. *Journal of Phytopathology* **139**, 229-237.

Fron, G. (1912). Contribution a l'étude de la maladie de 'pied noir des céréales' ou 'maladie du piétin'. *Annales de la Science Agronimique Francais,* **4**, 3-29.

Furuya, H. (1984). Occurrence of eyespot of wheat caused by *Pseudocercosporella herpotrichoides* (Fron) Deighton in Japan (*Triticum*). *Annals of the Phytopathological society of Japan* **50**, 77-81.

Gac, M.L., Monfort, F., Cavelier, N., Sailland, A. (1996a). Comparative study of morphological, cultural and molecular markers for the characterization of *Pseudocercosporella herpotrichoides* isolates. *European Journal of Plant Pathology* **102**, 325-337.

Gac, M.L., Montfort, F., Cavelier, N. (1996b). An assay based on the Polymerase Chain Reaction for the detection of N- and L-types of *Pseudocercosporella herpotrichoides* in wheat. *Journal of Phytopathology* **144,** 513-518.

Gac, M.L., Montfort, F., Cavelier, N., Hourmant, P. (1999). Value of the polymerase chain reaction for studying the development in the field of *Tapesia yallundae* and *Tapesia acuformis* and for evaluating effects of triticonazole seed treatment. *Journal of Phytopathology* **147**, 707-715.

Gale, M.D., Scott, P.R., Law, C.N., Ainsworth, C.C., Hollins, T.W., Worland, A.J. (1984). An α-amylase gene from *Aegilops ventricosa* transferred to bread wheat together with a factor for eyespot resistance. *Heredity* **52**, 431-435.

Gallimore, K., Knights, I.K., Barnes, G. (1987). Sensitivity of *Pseudocercosporella herpotrichoides* to the fungicide prochloraz. *Plant Pathology* **36**, 290-296.

Garrett, S. D. (1975). Cellulolysis rate and competitive saprophytic colonization of wheat straw by foot-rot fungi. *Soil Biology and Biochemistry* **7**, 323-327.

Glynn, N.C. (2002). Studies on the epidemiology and control of *Fusarium* seedling blight of wheat using PCR technologies. PhD Thesis, Open University, UK.

Glynne, M. D., Moore, J. (1949). Effect of previous crops on the incidence of eyespot on winter wheat. *Annals of Applied Biology* **30**, 341.

Glynne, M.D. (1936). Some new british records of fungi on wheat: *Cercosporella herpotrichoides* Fron., *Gibellina cerealis* Pass., and *Ophiobolus herpotrichus* (Fr.) Sacc. *Transactions of the British Mycological Society* **20**, 120-122.

Glynne, M.D. (1942). *Cercosporella herpotrichoides* Fron causing eyespot of wheat in Great Britain. *Annals of Applied Biology* **29**, 254-264.

Glynne, M.D. (1944). Eyespot, *Cercosporella herpotrichoides* Fron, and lodging of wheat. *Annals of Applied Biology* **31**, 377-378.

Glynne, M.D. (1953). Production of spores by *Cercosporella herpotrichoides*. *Transactions of the British Mycological Society* **36**, 46-51.

Glynne, M.D. (1963). Eyespot (*Cercosporella herpotrichoides*) and other factors influencing yield of wheat in the six-course rotation experiment at Rothamsted (1930-60). *Annals of Applied Biology* **51**, 189-214.

Glynne, M.D., Dion, W.M., Weil, J.W. (1945). The effect of eyespot (*Cercosporella herpotrichoides* Fron.) on wheat and the influence of nitrogen on the disease. *Annals of Applied Biology* **32**, 297-303.

Glynne, M.D., Salt, G.A. (1958). Eyespot of wheat and barley. Report of Rothamsted Experimental Station 1958, pp. 231-241.

Goulds, A., Fitt, B.D.L. (1988). The comparative epidemiology of eyespot (*Pseudocercosporella herpotrichoides*) types in winter cereal crops. Proceedings of the Brighton Crop Protection Conference. British Crop Protection Council, Farnham, UK, pp. 1035-1040.

Goulds, A., Fitt, B.D.L. (1990a). The development of eyespot on seedling leaf sheafs in winter wheat and winter barley crops inoculated with W-type and R-type isolates of *Pseudocercosporella herpotrichoides*. *Journal of Phytopathology* **130**, 161-173.

Goulds, A., Fitt, B.D.L. (1990b). Effects of fungicide timing on the severity of eyespot on winter wheat or winter barley inoculated with W-type or R-type isolates of *Pseudocercosporella herpotrichoides*. *Crop Protection* **9**, 265-270.

Goulds, A., Fitt, B.D.L. (1991a). Prediction of eyespot severity on winter wheat or winter barley inoculated with W-type or R-type isolates of *Pseudocercosporella herportichoides*. *Journal of Phytopathology* **132**, 105-115.

Goulds, A., Fitt, B.D.L. (1991b). The development of eyespot on stems of winter wheat and winter barley in crops inoculated with W-type or R-type isolates of *Pseudocercosporella herpotrichoides*. *Journal of Plant Diseases and Protection* **98**, 490-502.

Goulds, A., Polley, R.W. (1990). Assessment of eyespot and other stem base diseases of winter wheat and winter barley. *Mycological Research* **94**, 819-822.

Hardwick, N.V., Jones, D.R., Slough, J.E. (2001). Factors affecting diseases of winter wheat in England and Wales, 1989-98. *Plant Pathology* **50**, 453-462.

Higgins, S. (1984). Factors affecting spore germination and initial growth of *Pseudocercosporella herpotrichoides* on wheat. *Transactions of the British Mycological Society* **82**, 443-448.

Higgins, S., Fitt, B.D.L. (1984). Production and pathogenicity to wheat of *Pseudocercosporella herpotrichoides* conidia. *Journal of Phytopathology* **111**, 222-231.

Higgins, S., Fitt, B.D.L. (1985). Effects of water potential and temperature on the development of eyespot lesions in wheat. *Annals of Applied Biology* **107**, 1-9.

Higgins, S., Fitt, B.D.L., White, R.P. (1986). The development of eyespot (*Pseudocercosporella herpotrichoides*) lesions in winter wheat. *Journal of Plant Diseases and Protection* **93**, 210-220.

Hinton, M.J., Parry, D.W. (1993). Screening selected fungi for antagonism towards *Pseudocercosporella herpotrichoides* (Fron) Deighton, the cause of eyespot disease of cereals. *Biocontrol Science and Technology* **3**, 13-19.

Hoare, F.A., Hunter, T., Jordan, V.W.L. (1986). Influence of spray programmes on development of fungicide resistance in the eyespot pathogen of wheat, *Pseudocercosporella herpotrichoides*. *Plant Pathology* **35**, 505-511.

Hollins, T.W., Lockley, K.D., Blackman, J.A., Scott, P.R., Bingham, J. (1988). Field performance of Randezvous, a wheat cultivar with resistance to eyespot (*Pseudocercosporella herpotrichoides*) derived from *Aegilops ventricosa*. *Plant Pathology* **37**, 251-260.

Hollins, T.W., Scott, P.R. (1980). Epidemiology of eyespot (*Pseudocercosporella herpotrichoides*) on winter wheat, with particular reference to the period of infection. *Annals of Applied Biology* **95**, 19-29.

Hollins, T.W., Scott, P.R., Paine, J.R. (1985). Morphology, benomyl resistance and pathogenicity to wheat and rye of isolates of *Pseudocercosporella herpotrichoides*. *Plant Pathology* **34**, 369-379.

Hornby, D., Bateman, G.L., Gutteridge, R.J., Lucas, P., Osbourn, A.E., Ward, E., Yarham, D.J. (1998). Take-all and cereal production systems. In: Take-all disease of cereals, A regional perspective. (Ed. D. Hornby). CAB International, Wallingford, UK, pp.103-159.

Hughes, G., McRoberts, N., Burnett, F.J. (1999). Decision-making and diagnosis in disease management. *Plant Pathology* **48**, 147-153.

Humphreys, J., Cooke, B.M., Storey, T. (1995). Effects of seed-borne *Microdochium nivale* on establishment and grain yield of winter sown wheat. *Plant Varieties and Seeds* **8**, 107-117.

Hunter, T. (1989). Occurrence of *Tapesia yallundae*, teleomorph of *Pseudocercosporella herpotrichoides*, on unharvested wheat culms in England. *Plant Pathology* **38**, 598-603.

Jahier, J., Doussinault, G., Dosba, F., Bourgeois, F. (1979). Monosomic analysis of resistance to eyespot in the variety " Roazon ". Proceedings of the 5th International Wheat Genetics Symposium, New Delhi, pp. 437-440.

Jahier, J., Lucas, H. (1987). Study of the 7D chromosome of Roazon carrying *Aegilops ventricosa* genes for resistance to eyespot. European Wheat Aneuploid Co-op Newsletter 1987, 23-25.

Jalaluddin, M., Jenkyn, J.F. (1996). Effects of wheat crop debris on the sporulation and survival of *Pseudocercosporella herpotrichoides*. *Plant Pathology* **45**, 1052-1064.

Jenkyn, J.F., Gutteridge, R.J., Jalaluddin, M. (1994). Straw disposal and cereal diseases. In: Ecology of Plant Pathogens, (Eds J.P. Blakeman, B. Williamson), CAB International, Wallingford, UK, pp.285-300.

Jones, D.R. (1994). Evaluation of fungicides for control of eyespot disease and yield loss relationships in winter wheat. *Plant Pathology* **43**, 831-846.

Jones, D.R. (1995). Timing of fungicide application for control of eyespot disease in winter wheat. *Crop Protection* **14**, 247-256.

Jørgensen, J. (1964a). Some observations on the effect of temperature on the sporulation of *Cercosporella herpotrichoides* Fron. *Acta Agriculture Scandinavica* **14**, 126-128.

Jørgensen, J. (1964b). Investigations on loss in yield due to attack by *Cercosporella herpotrichoides* Fron in field experiments with winter wheat. *Acta Agriculture Scandinavica* **14**, 12-20.

Jørgensen, L.N., Secher, B.J.M., Nielsen, G.C. (1996). Monitoring diseases of winter wheat on both a field and national level in Denmark. *Crop Protection* **15**, 383-390.

Julian, A.M., Lucas, J.A. (1990). Isozyme polymorphism in pathotypes of *Pseudocercosporella herpotrichoides* and related species from cereals. *Plant Pathology* **39**, 178-190.

Kimber, G. (1967). The incorporation of the resistance of *Aegilops ventricosa* to *Cercosporella herpotrichoides* into *Triticum aestivum*. *Journal of Agricultural Science* **68**, 373-376.

King, A.C. (1990). First record of *Tapesia yallundae* as the teleomorph of *Pseudocercosporella herpotrichoides* var. *acuformis* , and its occurrence in the field in the Federal Republic of Germany. *Plant Pathology* **39**, 44-49.

King, J.E., Griffin, M.J. (1985). Survey of benomyl resistance in *Pseudocercosporella herpotrichoides* on winter wheat and barley in England and Wales in 1983. *Plant Pathology* **34**, 272-283.

Kryuchkova, L. (2000). Stem-base diseases in Ukraine. Proceedings of the Brighton Crop Protection Conference. British Crop Protection Council, Farnham, UK, pp. 113-118.

Lange-de la Camp, M. (1966). Die Wirkungsweise von *Cercosporella herpotrichoides* Fron, dem Erreger der Halmbruchkrankheit des Getreides. II Agressivität des Erregers. *Journal of Phytopathology* **56**, 155-190.

Law, C.N., Scott, P.R., Worland, A.J., Hollins, T.W. (1976). The inheritance of resistance to eyespot (*Cercosporella herpotrichoides*) in wheat. *Genetical Research: Cambridge* **25**, 73-79.

Leroux, P. (1998). Progress and problems in the control of cereal eyespot fungi in France. *Pesticide Outlook*, **August 1998**, 34-38.

Leroux, P., Albertini, C., Arnold, A., Gredt, M. (2003). Le piètin-verse des céréals: Caractéristiques et distribution des souches de *Tapesia acuformis* et *Tapesia yallundae* résistantes aux fungicides. *Phytoma* **557**, 8-13.

Leroux, P., Gredt, M. (1997). Evolution of fungicide resistance in the cereal eyespot fungi *Tapesia yallundae* and *Tapesia acuformis* in France. *Pesticide Science* **51**, 321-327.

Leroux, P., Marchegay, P. (1991). Resistance to prochloraz in strains of the cereal eyespot fungus *Pseudocercosporella herpotrichoides* isolated from winter wheat in France. *Agronomie* **11**, 767-776.

Lind, V. (1992). Measuring the severity of eyespot disease induced by *Pseudocercosporella herpotrichoides* (Fron) Deighton in wheat cultivars at different growth stages. *Plant Breeding* **108**, 202-209.

Lind, V., Züchner, S., Spanakakis, A., Thiele, A. (1994). Analyses of interactions of wheat cultivars in tests for resistance to *Pseudocercosporella herpotrichoides*. *Plant Breeding* **113**, 272-280.

Locke, T., Moon, L.M., Evans, J. (1987). Survey of benomyl resistance in *Fusarium* species on winter wheat in England and Wales in 1986. *Plant Pathology* **36**, 589-593.

Lucas, J.A., Dyer, P.S., Murray, T.D. (2000). Pathogenicity, host-specificity, and population biology of *Tapesia* spp., causal agents of eyespot disease of cereals. *Advances in Botanical Research* **33**, 225-258.

Lucas, P., Monfort, F., Cavelier, N, Cavelier, A. (1988). Attempts to combine different methods of control of foot and root diseases of winter wheat. In: Proceedings of a meeting of the EC Experts Group. Integrated Crop Protection in Cereals, Littlehampton, pp. 237-249.

Lupton, F.G.H., Macer, R.C.F. (1955). Winter wheats resistant to eyespot. *Agriculture* **62**, 54-56.

Lupton, F.G.H., Macer, R.C.F. (1958). The breeding of disease resistant winter wheats. *Agriculture* **64**, 540-544.

Macer, R.C.F. (1961a). The survival of *Cercosporella herpotrichoides* Fron in winter straw. *Annals of Applied Biology* **49**, 165-172.

Macer, R.C.F. (1961b). Saprophytic colonization of wheat straw by *Cercosporella herpotrichoides* Fron and other fungi. *Annals of Applied Biology* **49**, 152-164.

Macer, R.C.F. (1966). Resistance to eyespot disease (*Cercosporella herpotrichoides* Fron) determined by a seedling test in some forms of *Triticum*, *Aegilops*, *Secale* and *Hordeum*. *Journal of Agricultural Science* **67**, 389-396.

Maia, N. (1967). Obtention de bles tendres resistants au pietin-verse par croisements interspecifiques bles X *Aegilops*. *Comptes Rendus Hebdomadaires des Seamces de l'Acedemie d'Agriculture de France* **53**, 149-154.

Maraite, H., Delforge, C., Meunier, S., Tetelain, A. (1985). Distribution of MBC -resistance in *Pseudocercosporella herpotrichoides* on winter wheat in Belgium. *Mededelingen van de Faculteit Lanndbouwwetenschappen Rijksununiversite.* **50**, 1173-1180.

Mauler-Machnik, A., Rosslenbroich, H-J., Dutzmann, S., Applegate, J., Jautelat, M. (2002). JAU 6476-a new dimension DMI fungicide. In: Proceedings of the Brighton Crop Protection Conference. British Crop Protection Council, Farnham, UK, pp. 389-394.

Mbwaga, A.M., Menke G., Grossman, F. (1997). Investigations on the activity of cell-wall degrading enzymes in young wheat plants after infection with *Pseudocercosporella herpotrichoides* (Fron) Deighton. *Journal of Phytopathology* **145**, 123-130.

McKay, R., Loughnane, J.B., Kavanagh, T. (1956). Virulent attacks of eyespot on oats. *Nature* **177**, 193.

McKinney, H.H. (1925). Foot- rot diseases of wheat in America. *United States Department of Agriculture Bulletin* **1347**, 1-40.

McMillin, D.E., Allan, R.E., Roberts, D.E. (1986). Association of an isozyme locus and strawbreaker foot rot resistance derived from *Aegilops ventricosa* in wheat. *Theoretical and Applied Genetics* **72,** 743-747.

Mielke, H. (1983). Untersuchungen über den Einfluβ verschiedener Bodenbearbeitungen auf Fuβkrankheiten des Getreides. *Nachrichtenbl dtsch Pflanzenschutzdienstes, Braunschweig* **35**, 33-39.

Millar, C.S., Colhoun, J. (1969). Fusarium diseases of cereals. VI. Epidemiology of *Fusarium nivale* on wheat. *Transactions of the British Mycological Society* **52**, 192-204.

Moreau, J.M., Maraite, H. (1996). Evidence for a heterothallic mating system in *Tapesia acuformis* using benomyl sensitivity and esterase isoenzyme profiles. *Mycological Research* **100**, 1227-1236.

Moreau, J.M., Van Schingen, J.C., Maraite, H. (1989). Detection of *Tapesia yallundae*, the teleomorph of *Pseudocercosporella herpotrichoides* on wheat stubles in Belgium. *Mededelingen van de Faculteit Lanndbouwwetenschappen Rijksununiversite* **54**, 555-559.

Morgan, L.W., Bateman, G.L., Edwards, S.G., Marshall, J., Nicholson, P., Nuttall, M., Parry, D.W., Scrancher, M., Turner, A.S., (1998). Fungicide evaluation and risk assessment of wheat stem-base diseases using PCR. In: Proceedings of the Brighton Crop Protection Conference. British Crop Protection Council, Farnham, UK, pp.1011-1016.

Muranty, H., Jahier, J., Tangui, A.-M., Worland, A.J., Law, C. (2002). Inheritance of resistance of wheat to eyespot at the adult stage. *Plant Breeding* **121**, 536-538.

Murray, G.M., Heenan, D.P., Taylor, A.C. (1991). The effect of rainfall and crop management on take-all and eyespot of wheat on the field. *Australian Journal of Experimental Agriculture* **31,** 645-651.

Murray, T.D., Bruehl, G.W. (1983). Role of hypodermis and secondary cell wall thickening in basal stem internodes in resistance to strawbreaker foot rot in winter wheat. *Phytopathology* **73**, 261-268.

Murray, T.D., de la Peña, R.C., Yildirim, A., Jones, S.S. (1994). A new source of resistance to *Pseudocercosporella herpotrichoides*, cause of eyespot disease of wheat, located on chromosome 4V of *Dasypyrum villosum*. *Plant Breeding* **113**, 281-286.

Nicholson, P., Dyer, P.S., Rezanoor, H.N., Walkowial-Cagara, I., Hollins, T.W. (1995). Mating relationships between different pathotypes of *Tapesia yallundae*, the teleomorph of the eyespot pathogen *Pseudocercosporella herpotrichoides*. *Mycological Research* **99**, 457-465.

Nicholson, P., Hollins, T.W., Rezanoor, H.N. (1991). A comparison of cultural, morphological and DNA markers for the classification of *Pseudocercosporella herpotrichoides*. *Plant Pathology* **40**, 584-594.

Nicholson, P., Parry, D.W. (1996). Development of a PCR assay to detect *Rhizoctonia cerealis*, the cause of sharp eyespot in wheat. *Plant Pathology* **45**, 872-883.

Nicholson, P., Rezanoor, H.N. (1994). The use of random amplified polymorphic DNA to identify pathotype and detect variation in *Pseudocercosporella herpotrichoides*. *Mycological Research* **98**, 13-21.

Nicholson, P., Rezanoor, H.N., Hollins, T.W. (1993). Classification of a world-wide collection of isolates of *Pseudocercosporella herpotrichoides* by RFLP analysis of mitochondrial and ribosomal DNA and host range. *Plant Pathology* **42**, 58-66.

Nicholson, P., Rezanoor, H.N., Parry, D.W. (1996). Detection and quantification of individual fungal species in disease complexes by polymerase chain reaction (PCR). In: Proceedings of the Brighton Crop Protection Conference. British Crop Protection Council, Farnham, UK, pp. 227-232.

Nicholson, P., Rezanoor, H.N., Simpson, D.R., Joyce, D. (1997). Differentiation and quantification of the cereal eyespot fungi *Tapesia yallundae* and *Tapesia acuformis* using a PCR assay. *Plant Pathology* **46**, 842-856.

Nicholson, P., Turner, A.S. (2000). Cereal stem-base disease - a complex issue. In: Proceedings of the Brighton Crop Protection Conference. British Crop Protection Council, Farnham, UK, pp.99-106.

Nicholson, P., Turner, A.S., Edwards, S.G., Bateman, G.L., Morgan, L.W., Parry, D.W., Marshall, J., Nuttall, M. (2002). Development of stem-base pathogens on different cultivars of winter wheat determined by quantitative PCR. *European Journal of Plant Pathology* **108**, 163-177.

Nirenberg, H.I. (1981). Differentiation of *Pseudocercosporella* strains causing foot rot disease of cereals. I. Morphology. *Journal of Plant Diseases and Protection* **88**, 241-248.

Oort, A.J.P. (1936). The eyespot disease of cereals, caused by *Cercosporella herpotrichoides* Fron. *Tijdschrift Over Plantenziekten* **42**, 180-233.

Parry, D.W. (1990). The incidence of *Fusarium* spp. in stem bases of selected crops of winter wheat in the Midlands, UK. *Plant Pathology* **39**, 619-622.

Parry, D.W., Jenkinson, P., McLeod, L. (1995). Fusarium ear blight (scab) in small grain cereals - A review. *Plant Pathology* **44**, 207-238.

Parry, D.W., Pettitt, T.R., Jenkinson, P., Lees, A.K. (1994). The cereal *Fusarium* complex. In: Ecology of Plant Pathogens, (Eds. J.P. Blakeman, B. Williamson), CAB International, Wallingford, UK, pp.301-320.

Pettitt, T., Parry, D.W., Polley, R.W. (1993). Improved estimation of the incidence of *Microdochium nivale* in winter wheat stems in England and Wales, during 1992, by use of benomyl agar. *Mycological Research* **97**, 1172-1174.

Pinthus, M.J. (1973). Lodging in wheat, barley and oats: the phenomenon, its causes, and preventative measures. *Advances in Agronomy* **25**, 209-263.

Polley, R.W., Clarkson, J.D.S. (1978). Forecasting cereal disease epidemics. *Plant Disease Epidemiology* **1978**, 141-150.

Polley, R.W., Turner, J.A. (1995). Surveys of stem-base diseases and fusarium ear diseases in winter wheat in England, Wales and Scotland, 1989-1990. *Annals of Applied Biology* **126**, 45-59.

Ponchet, J. (1959). La maladie du pietin-verse des cereales: *Cercosporella herpotrichoides* Fron. Importance agronomique, biologie, epiphytologie. *Annales des Epiphyties* **I** 45-98.

Poupard, P., Cavalier, N. (1992). Variability of *Pseudocercosporella herpotrichoides* (Fron) Deighton, the cause of eyespot in cereals: A review. *Agronimie* **12**, 205-218.

Poupard, P., Grare, S., Cavelier, N., Lind, V. (1994). Development of *Pseudocercosporella herpotrichoides* (Fron) Deighton var. *herpotrichoides* and var. *acuformis* on wheat plants measured by ELISA. *Journal of Phytopathology* **140,** 301-311.

Poupard, P., Simonet, P., Cavelier, N., Bardin, R. (1993). Molecular characterization of *Pseudocercosporella herpotrichoides* isolates by amplification of ribosomal DNA internal transcribed spacers. *Plant Pathology* **42**, 873-881.

Prew, R.D., Ashby, J.E., Bacon, E.T.G., Christian, D. G., Gutteridge, R.J., Jenkyn, J.F., Powell, W., Todd, A.D. (1995). Effects of incorporating or burning straw, and of different cultivation systems, on winter wheat grown on two soil types, 1985-91. *Journal of Agricultural Science, Cambridge* **124**, 173-194.

Prew, R.D., McIntosh, A.H. (1975). Effects of benomyl and other fungicides on take-all, eyespot and sharp eyespot diseases of winter wheat. *Plant Pathology* **24**, 67-71.

Priestley, R.A., Dewey, F.M. (1993). Development of a monoclonal antibody immunoassay for the eyespot pathogen *Pseudocercosporella herpotrichoides*. *Plant Pathology* **42**, 403-412.

Priestley, R.A., Dewey, F.M., Nicholson, P., Rezanoor, H.N. (1992). Comparison of isoenzyme and DNA markers for differentiating W-, R- and C- pathotypes of *Pseudocercosporella herpotrichoides*. *Plant Pathology* **41**, 591-599.

Rapilly, F., Laborie, Y., Eschenbrenner, P., Chisnel, E., Lacrose, F. (1979). La prévision du piétin-verse sur blé d'hiver. *Perspectives Agricoles* **23**, 30-40.

Reinecke, P., Duben, J., Fehrmann, H. (1979). Antagonism between fungi of the foot rot complex of cereals. In: Soil-Borne Plant Pathogens. (Eds. B. Shippers and W. Gams). Academic press, London, pp 321-328.

Reinecke, P., Fokkema, N.J. (1979). *Pseudocercosporella herpotrichoides*: Storage and mass production of conidia. *Transactions of the British Mycological Society* **72**, 329-331.

Reinecke, P., Fokkema, N.J. (1981). An evaluation of methods of screening fungi from the haulm base of cereals for antagonism to *Pseudocercosporella herpotrichoides* in wheat. *Transactions of the British Mycological Society* **77**, 343-350.

Robbertse, B., Campbell, G.F., Crous, P.W. (1995). Revision of *Pseudocercosporella*-like species causing eyespot disease of wheat. *South African Journal of Botany* **61**, 43-48.

Robbertse, B., Crous, P. W., Holz, G. (1994). *Tapesia yallundae* collected from wheat stubble in South Africa. *Mycopathologia* **125**, 23-28.

Robbertse, B., Holz, G., Crous, P.W. (1996). Sensitivity of South African *Ramulispora herpotrichoides* isolates to carbendazim and ergosterol biosynthesis inhibitors. *Plant Pathology* **45**, 270-275.

Rowe, R.C., Powelson, R.L. (1973a). Epidemiology of *Cercosporella* footrot of wheat: Spore production. *Phytopathology* **63**, 981-984.

Rowe, R. C., Powelson, R. L. (1973b). Epidemiology of *Cercosporella* footrot of wheat: Disease spread. *Phytopathology* **63**, 984-988.

Royle, D.J. (1998). The importance and control of stem base diseases of cereals. HGCA Project Report No.176. Home Grown Cereals Authority, London, pp. 20-30.

Sanders, P.L., De Waard, M.A., Loerakker, W.M. (1986). Resistance to carbendazim in *Pseudocercosporella herpotrichoides* from Dutch wheat fields. *Netherlands Journal of Plant Pathology* **92**, 15-20.

Sanderson, F.R. & King, A. C. (1988). Field occurrence of *Tapesia yallundae*, the teleomorph *Pseudocercosporella herpotrichoides*. *Australasian Plant Pathology* **17**, 20-21.

Saxby, S.H. (1943). Eye-spot in wheat. *The New Zealand Journal of Agriculture* **66**, 257-261.

Scott, D.B. (1996). Disease control and yield improvement obtained with fungicide applications to spring wheat in South Africa. *Crop Protection* **15**, 167-170.

Scott, P.R. (1971). The effect of temperature on eyespot (*Cercosporella herpotrtichoides*) in wheat seedlings. *Annals of Applied Biology* **68**, 169-175.

Scott, P.R. and Hollins, T.W. (1977). Interactions between cultivars of wheat and isolates of *Cercosporella herpotrichoides*. *Transactions of the British Mycological Society* **69**, 397-403.

Scott, P.R., Defosse, L., Vandam, J., Doussinault, G. (1976). Infection of lines of *Triticum*, *Secale*, *Aegilops* and *Hordeum* by isolates of *Cercosporella herpotrichoides*. *Transactions of the British Mycological Society* **66**, 205-210.

Scott, P.R., Hollins, T.W. (1974). Effects of eyespot on the yield of winter wheat. *Annals of Applied Biology* **78**, 269-279.

Scott, P.R., Hollins, T.W. (1978). Prediction of yield loss due to eyespot in winter wheat. *Plant Pathology* **27**, 125-131.

Scott, P.R., Hollins, T.W. (1980). Pathogenic variation in *Pseudocercosporella herpotrichoides*. *Annals of Applied Biology* **94**, 297-300.

Scott, P.R., Hollins, T.W. (1982). Eyespot. Annual Report for 1981. Plant Breeding Institute, Cambridge, pp. 96-98.

Scott, P.R., Hollins, T.W., Muir, P. (1975). Pathogenicity of *Cercosporella herpotrichoides* to wheat, barley, oats and rye. *Transactions of the British Mycological Society* **65**, 529-538.

Scott, P.R., Hollins, T.W., Summers, R.W. (1989). Breeding for resistance to two soil-borne diseases of cereals. *Vortrage Fur Pflanzenzuchstung* **16**, 217-230.

Slopek, S.W., Labun, T.J. (1990). Incidence of eyespot in barley in Alberta, 1989. *Inventaire des maladies des plantes au Canada* **70**, 36.

Smiley, R.W., Collins, H.P., Rasmussen, P.E. (1996). Diseases of wheat in long-term agronomic experiments at Pendleton, Oregon. *Plant Disease* **80**, 813-820.

Smith, J.M., Cook, S.K., Mills, A.R., Bacon, E.T.C., Clarke, J.H. (2000). The effect of three or five years set-aside on the husbandry and grain yield of subsequent cereal crops in the UK. *Plant and Soil* **225**, 279-297.

Soleimani, M.J., Deadman, M.L., McCartney, H.A. (1996). Splash dispersal of *Pseudocercosporella herpotrichoides* spores in wheat monocrop and wheat-clover bicrop canopies from simulated rain. *Plant Pathology* **45**, 1065-1070.

Sprague, R. (1931). *Cercosporella herpotrichoides* Fron, the cause of columbia basin footrot of winter wheat. *Science* **74**, 51-53.

Sprague, R. (1936). Relative susceptability of certain species of Graminae to *Cercosporella herpotrichoides*. *Journal of Agricultural Research* **53**, 659-670.

Sprague, R., Fellows, H. (1934). *Cercosporella* foot rot of winter cereals. Technical Bulletin No. 428, US Department of Agriculture, Washington, D.C, 1-24.

Stadnik, M.J., Buchenauer, H. (1999). Control of wheat diseases by benzpthidiazole-derivative and modern fungicides. *Journal of Plant Diseases and Protection* **106**, 466-475.

Stewart, E. L., Zhaowei, L., Crous, P.W., Szabo, L. (1999). Phylogenetic relationships among some cercosporoid anamorphs of *Mycosphaerella* based on rDNA sequence analysis. *Mycological Research* **103**, 1491-1499.

Strausbaugh, C.A., Murray, T.D. (1989a). Inheritance of resistance to *Pseudocercosporella herpotrichoides* in three cultivars of winter wheat. *Phytopathology* **79**, 1048-1053.

Strausbaugh, C.A., Murray, T.D. (1989b). Use of epidermal cell responses to evaluate resistance of winter wheat cultivars to *Pseudocercosporella herpotrichoides*. *Phytopathology* **79**, 1043-1047.

Takeuchi, T., Kuninaga, S. (1994). Genetic relationships in *Pseudocercosporella herpotrichoides* determined from DNA relatedness. *Mycological Research* **98**, 1059-1064.

Takeuchi, T., Kuninaga, S. (1996). Determination of relationships in *Pseudocercosporella herpotrichoides* by analysis of mitochondrial DNA. *Mycological Research* **100**, 693-701.

Thomas, D, Maraite, H., Boutry, M. (1992). Identification of rye- and wheat -types of *Pseudocercosporella herpotrichoides* with DNA probes. *Journal of General Microbiology* **138**, 2305-2309.

Turner, A.S., Nicholson, P., Edwards, S.G., Bateman, G.L., Morgan, L.W., Todd, L.D., Parry, D.W., Marshall, J., Nutall, M. (2001). Evaluation of diagnostic and quantitative PCR for the identification and severity assessment of eyespot and sharp eyespot in winter wheat. *Plant Pathology* **50**, 463-469.

Turner, A.S., Nicholson, P., Edwards, S.G., Bateman, G.L., Morgan, L.W., Todd, A.D., Parry, D.W., Marshall, J., Nuttall, M. (2002). Relationship between brown foot rot and DNA of *Microdochium nivale*, determined by quantitative PCR, in stem bases of winter wheat. *Plant Pathology* **51**, 464-471.

Turner, A.S., O'Hara, R.B., Rezanoor, H.N., Nutall, M., Smith, J.N., Nicholson, P. (1999). Visual disease and PCR assessment of stem base diseases in winter wheat. *Plant Pathology* **48**, 742-748.

Uslu, E., Miller, T.E., Rezanoor, N.H., Nicholson, P. (1998). Resistance of *Dasypyrum villosum* to the cereal eyespot pathogens *Tapesia yallundae* and *Tapesia acuformis*. *Euphytica* **103**, 203-209.

Van Der Spek, J. (1975). The primary infection of wheat by *Cercosporella herpotrichoides* Fron. *Mededelingen van de Faculteit Lanndbouwwetenschappen Rijksununiversiteit* **40**, 587-595.

Van Der Spek, J., Maenhout, C.A., Ten Hag, B.A. (1974). Foot rot of wheat in the Netherlands and the chemical control of eyespot. *Mededelingen van de Faculteit Lanndbouwwetenschappen Rijksununiversiteit* **39**, 925-933.

Vilich, V. (1993). Crop rotation with pure stands and mixtures of barley and wheat to control stem and root rot diseases. *Crop Protection* **12**, 373-379.

Vilich-Meller, V. (1992). *Pseudocercosporella herpotrichoides*, *Fusarium* spp. and *Rhizoctonia cerealis* stem rot in pure stands and interspecific mixtures of cereals. *Crop Protection* **11**, 45-50.

Vincent, A., Ponchet, J., Koller, J. (1955). Recherche de varietes de bles tendres peu sinsibles au pieten-verse: Resultats preliminaires. *Annales de l'amelioration des plantes* **III**, 459-472.

Wallwork, H. (1987). A *Tapesia teleomorph* for *Pseudocercosporella herpotrichoides*, the cause of eyespot of wheat. *Australian Plant Pathology* **16**, 92-93.

Wallwork, H., Spooner, B. (1988). *Tapesia yallundae* - the teleomorph of *Pseudocercosporella herpotrichoides*. *Transactions of the British Mycological Society* **91**, 703-705.

West, S.J.E., Booth, G.M., Beck, J.J., Etienne, L. (1998). A survey of *Tapesia yallundae* and *Tapesia acuformis* in UK winter wheat crops using a polymerase chain reaction diagnostic assay. In: Proceedings of the Brighton Crop Protection Conference. British Crop Protection Council, Farnham, UK, pp. 1029-1034.

Wood, H.M., Dickinson, M.J., Lucas, J.A., Dyer, P.S. (2001). Cloning of the *CYP51* gene from the eyespot pathogen *Tapesia yallundae* indicates that resistance to the DMI fungicide prochloraz is not related to sequence changes in the gene encoding the target site enzyme. *FEMS Microbiology Letters* **196**, 183-187.

Worland, A.J., Law, C.N. (1985). Resistance to eyespot. Annual Report for 1985. Plant Breeding Institute, Cambridge, pp. 62-63.

Worland, A.J., Law, C.N., Hollins, T.W., Koebner, R.M.D., Guira, A. (1988). Location of a gene for resistance to eyespot (*Pseudocercosporella herpotrichoides*) on chromosome 7D of bread wheat. *Plant Breeding* **101**, 43-51.

Yarham, D.J., Norton, J. (1981). Effects of cultivation methods on disease. In: Strategies for the control of cereal disease, (Eds. J.F. Jenkyn, R.T. Plumb). Academic Press, London, UK, pp.157-166.

Yildirim, A., Jones, S.S., Murray, T.D. (1998). Mapping a gene conferring resistance to *Pseudocercosporella herpotrichoides* on chromosome 4V of *Dasypyrum villosum* in a wheat background. *Genome* **41**, 1-6.

Yildirim, A., Jones, S.S., Murray, T.D., Cox, T.S., Line, R.F. (1995). Resistance to stripe rust and eyespot diseases of wheat in *Triticum tauschii*. *Plant Disease* **79**, 1230-1236.

Zadoks, J.C., Chang, T.T., Konzak, C.F. (1974). A decimal code for the growth stages of cereals. *Weed Research* **14**, 415-421.

APPENDICES

Appendix 1. Artificial media.

Artificial media and conditions used for growing of cultures.

Medium	Contents per litre	Incubation conditions
Potato Dextrose Agar (PDA)	Potato infusion - 4g Glucose – 20g Agar – 15g	20° C, NUV light
Malt Yeast Glucose Agar (MYG)	Malt extract – 5g Yeast extract – 2.5g Glucose – 10g Agar – 20g	20° C, NUV light
Maize Agar (MA)	Cornmeal – 40g Agar –20g	13° C, 20° C, NUV light
Tap Water Agar (TWA)	Agar – 20g Tap water – 1000 ml	13° C, 20° C, NUV light
Synthetic Nutrient Agar (SNA)	KH_2PO_4 –1g KNO_3 –1g $MgSO_4$. $7H_2O$ – 0.5g KCl – 0.5g Glucose – 0.2g Sucrose – 0.2g Agar – 20g	13° C, NUV light
2% PDA	0.78g PDA 15g Agar	10° C, NUV light

NUV–near ultra violet

Appendix 2. Disease assessments for eyespot, sharp eyespot and brown foot rot.

Eyespot (*Oculimacula* spp.) and Sharp Eyespot (*Rhizoctonia cerealis*):

Healthy (Score 0) – No visible lesions on the stem.

Slight (Score 1) – One tiller or the main shoot showing symptoms (lesions girdling less than half the tiller circumference) of the plant.

Moderate (Score 2) – More than one tiller or the main shoot of the plant with lesions girdling less than half the tiller circumference **or** lesions girdling more than half the tiller circumference.

Severe (Score 3) – One tiller or the main shoot of the plant with at least one leaf sheath penetrated (GS 31) or lesions girdling more than half the stem circumference, tissue softened.

Brown foot rot (*Fusarium* spp.):

Clean (Score 0) – No visible browning of the stem.

Slight (Score 1) – One tiller or main shoot of the plant showing browning of the stem.

Moderate (Score 2) – One tiller or main shoot of the plant with at least one leaf sheath penetrated **or** more than one tiller showing browning of the stem.

Severe (Score 3) – All tillers and the main shoot with brown stems and more than one tiller or main shoot with penetrated leaf sheaths.

Appendix 3. Buffers used for DNA extraction and PCR

CTAB extraction buffer (g l^{-1})

87.7	Sodium chloride (Lancaster Synthesis Ltd, UK)
25.0	L – sorbose (Sigma)
10.0	Polyvinylpolypyrrolidone (PVPP) (Sigma)
10.0	N – lauroylsarcosine sodium salt (Sigma)
8.0	N – Hexadecyltrimethyl ammonium bromide (CTAB) (Sigma)
8.0	Ethylenediamine tetraacetic acid (EDTA) (Sigma)

TE buffer

10mM	Tris-HCl (pH 7.4) (Sigma)
1mM	EDTA (pH 8.0) (Sigma)

TAE buffer

0.04M	Tris-Acetate (Sigma)
0.001M	EDTA

Chelex extraction buffer (g per 20 ml SDW)

1	Chelex 100 (imnodiacetic acid) (Sigma)
0.25	Activated charcoal (Sigma)

Appendix 4. PCR reaction ingredients at working concentrations for diagnostic PCR (25 µl reaction volume) and quantitative PCR (50 µl reaction volume).

Nucleotides (1 or 2 µl added)
100 µM of each deoxynucleotide triphosphate (dCTP, dATP, dGTP, dTTP) (Abgene, UK)

PCR Buffer (3.5 or 7 µl added)

1.5 mM	Magnesium chloride (Sigma)
10 mM	Tris-HCl (Sigma)
50 mM	Potassium chloride (Sigma)
100 µg ml^{-1}	Gelatine (Sigma)
0.05%	Tween 20 (Sigma)
0.05%	Nonidet P-40 (Sigma)
5%	Glycerol
40 mg ml^{-1}	Cresol red

Primers (0.05 or 0.1 µl added)
100nM each forward and reverse primer

Polymerase enzyme (0.1 or 0.2 µl added)
0.5 units/25 µl Taq DNA polymerase (New England BioLabs, UK)

Ultra purified water (Severn Biotech Ltd, UK)
15.3 µl diagnostic PCR
20.6 µl quantitative PCR

Appendix 5. Primer details.

Species	Name/ direction	Sequence	Annealing Temp. °C	Genomic Region
Allium cepa	JBW/FL	TAC TTG GTA GTG GCA GCA GCA CAA	38°C(A)	n/a
Allium cepa	JBW/RL	AGG CCG CGA GTG GCG AAG CAG ACC	38°C(A)	n/a
Allium cepa	JBR/FL	ACT TCG GTA AGT TGC TCA TGC CCC	38°C(A)	n/a
Allium cepa	JBR/FR	AGG CCG CGA AAA GTG CCC TCC GTG	38°C(A)	n/a
Allium cepa	JBSE/FL	TTT AGA CGG TGG AAA TGC AGC GGA	38°C(A)	n/a
Allium cepa	JBSE/RL	ATT GAT ATG CCA TGG GGA TGG AGG	38°C(A)	n/a
O. yallundae	JBW/F	GGG GGC TAC CCT ACT TGG TAG	72°C(B)	rDNA
O. yallundae	JBW/R	CCA CTG ATT TTA GAG GCC GCG AG	72°C(B)	rDNA
O. acuformis	JBR/F	GGG GGC CAC CCT ACT TCG GTA A	72°C(B)	rDNA
O. acuformis	JBR/R	CCA CTG ATT TTA GAG GCC GCG AA	72°C(B)	rDNA
R. cerealis	JBSE/F	TGT GCA CCT GTT TAG ACG GT	62°C(C)	rDNA
R. cerealis	JBSE/R	TCC TCC GCT TAT TGA TAT GC	62°C(C)	rDNA
M. nivale	JB612/F	GGT GCT GTC TCT CGG GAC	60 °C(D)	rDNA
M. nivale	ITS-4/R	TCC TCC GCT TAT TGA TAT GC	60 °C(D)	rDNA
Fusarium spp.	JB566/F	GTT TTT AGT GGA ACT TCT GAG T	60 °C(D)	rDNA
Fusarium spp.	JB572/R	AAG TTG GGG TTT AAC GGC	60 °C(D)	rDNA
Allium cepa	ONI/F	TGC TCT GCT GAT GTT GCC AG	58°C(A)	Alliinase gene
Allium cepa	ONI/R	TAC ATG GGG ATG GAG GTC	58°C(A)	Alliinase gene

Underlined bases show bases in linker primers which are part of the original primer sequences
A-D in brackets show programmes used (Appendix 6)

Appendix 6. PCR programmes used.

Programme A

Step	Temperature °C	Time (s)
1	95	90
2	95	30
3	Anneal temp. for primer (Appendix 5)	20
4	72	40
Repeat from step two 20 times		
5	95	20
6	50	20
7	72	80
Repeat from step five 10 times		

Programme B

Step	Temperature °C	Time (s)
1	94	105
2	94	15
3	72	60
Repeat from step two 34 times		
4	72	300

Programme C

Step	Temperature °C	Time (s)
1	94	105
2	94	15
3	62	15
4	72	45
Repeat from step two 30 times		
4	72	300

Programme D

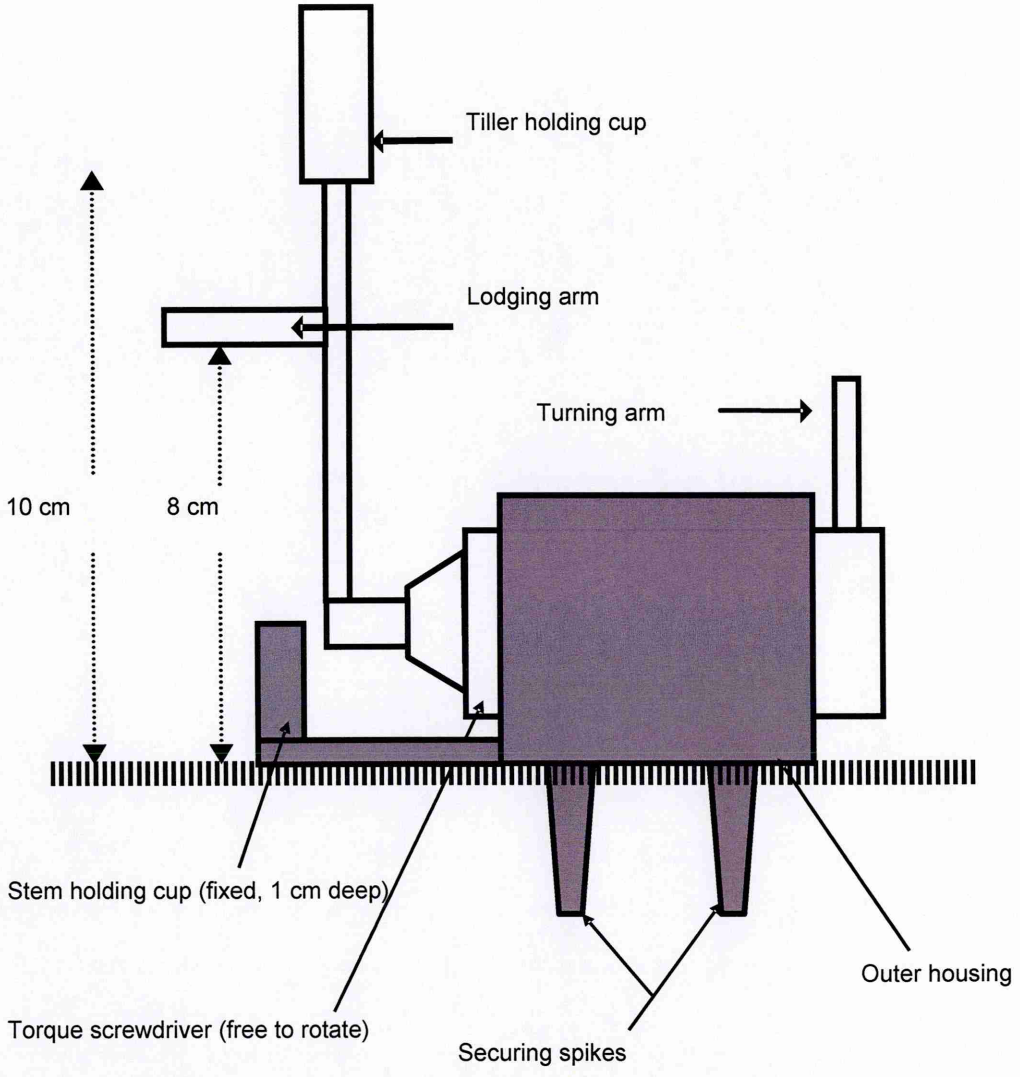
Step	Temperature °C	Time (s)
1	94	105
2	94	15
3	60	15
4	72	45
Repeat from step two 34 times		
4	72	300

Appendix 7. Cultivation operations carried out on inoculated experiment, Consort (Tibberton), 2001.

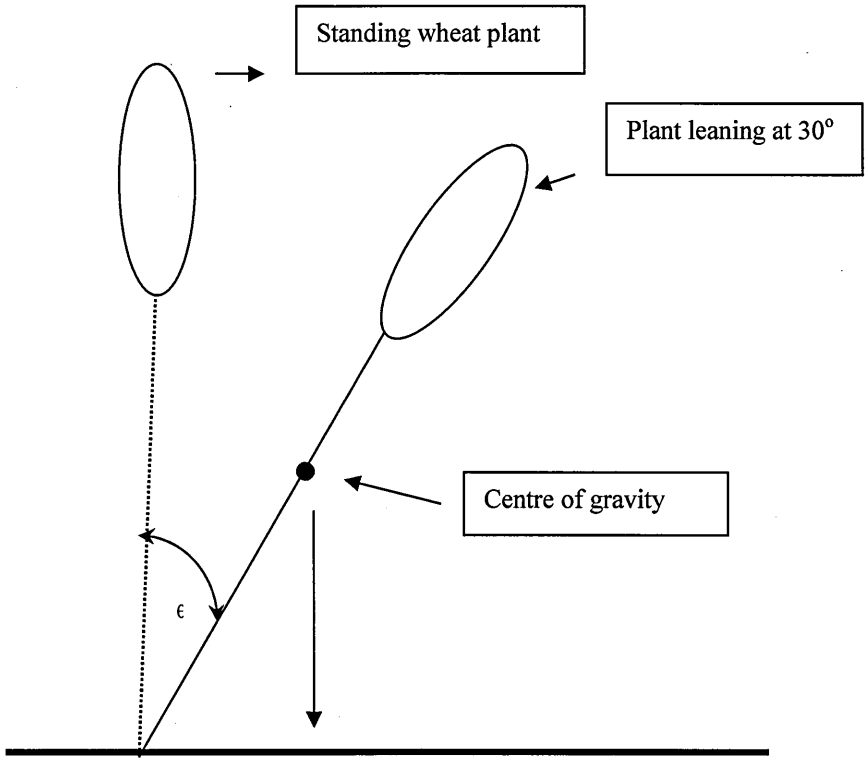
Consort, 2001

Date	Operation/ substance	Common name of product	Active ingredient g l^{-1} or kg^{-1}	Applied field rate l or kg ha^{-1}
15.10.01	Ploughed			
02.11.01	Power harrowed			
02.11.01	Drilled	Consort		196
09.04.02	Fungicide	Folicur (tebuconazole)+ Tern (fenpropidin)+Amistar (azoxystrobin)	100+750+125	0.4+1+0.5
17.04.02	Nutrient	Manifol (Mn)+Manganese sulphate (Mn SO_4)	500+96	1 3
02.05.03	Herbicide	Ally (metsulforon-methyl)	20	0.002
02.05.03	Nutrient	Manganese sulphate ($MnSO_4$)+Coptrel (Cu)	128+250	4+0.5
10.04.03	Fertiliser	Crop Master	28N:5P:5K:6SO_3	70:13:13:15
10.05.02	Fertiliser	Crop Master	28N:5P:5K:6SO_3	40:7:7:9
22.05.02	Fertiliser	Crop Master	28N:5P:5K:6SO_3	53:10:10:11
01.06.02	Nutrient	Manifol (Mn)	500	1
01.06.02	Herbicide	Starane (fluroxypyr)	200	1

Appendix 8. Plan of the lodging meter (not to scale. Crook & Ennos, 2000). The torque screwdriver is free to rotate within its housing (by use of the turning arm) causing the lodging arm to rotate, thereby pushing the plant over (not shown). Securing spikes ensure that the outer casing remains stationary during the tests.



Appendix 9. Simplified diagram of lodging.



Appendix 10. Cultivation operations carried out on Consort, Savannah and Claire.

Consort, 2000

Date	Operation/ substance	Common name of product	Active ingredient g l^{-1}/kg^{-1}	Applied field rate l or kg ha^{-1}
08.09.00	Ploughed Power harrowed			
08.09.00	Drilled	Consort		79
16.10.00	Herbicide	Encore (isoproturon + pendimethalin)	125+250	4
03.03.01	Fertiliser	Kemira 4	15N:15P:20K	56:56:75
09.04.01	Fertiliser	Kayennes	25N:0P:13K	65:0:34
11.04.01	Herbicide	Boxer (florasulam)	50	0.08
23.05.01	Fungicide	Mantra (epoxiconazole + fenpropimorph + kresoxim-methyl)	125+150+125	0.7

Savannah, 2000

Date	Operation/ substance	Common name of product	Active ingredient g l^{-1}/kg^{-1}	Applied field rate l or kg ha^{-1}
01.09.00	Ploughed Power harrowed			
01.09.00	Drilled	Savannah		150
01.03.01	Herbicide	Capture (bromixynil + diflufenican + ioxinil)	300+50+200	1
03.03.01	Fertiliser	Nitraprill	34.5N	69
12.06.01	Fungicide	Folicur (tebuconazole) + Amistar (azoxystrobin)	250+250	0.5 + 0.5

Claire, 2001

Date	Operation/ substance	Common name of product	Active ingredient g l^{-1}/kg^{-1}	Applied field rate l or kg ha^{-1}
14.09.01	Ploughed Power harrowed			
14.09.01	Drilled	Claire		110
30.09.01	Herbicide	Aligran (isoproturon)	83	3
30.09.01	Herbicide	Ingot (diflufenican + flurtamone + isoproturon)	27+67+400	1.5
30.09.01	Nutrient	Sumi-Alpha (esfenvalerate)	25	0.1
16.03.02	Fertiliser	Kemira 4	15N:15P:20K	58:58:77
08.04.02	Herbicide	Stamina (alkoxylated fatty amine) + Boxer (florasulam)	100+50	2.5+0.1
08.04.02	Herbicide	Biplay (metsulfuron-methyl + tribenuron-methyl)	13+26.1	0.03
09.04.02	Fertiliser	Nitraprill	34.5N	70
15.04.02	Fertiliser	Nitraprill	34.5N	85
06.05.02	Fungicide	Mantra (epoxiconazole + fenpropimorph + kresoxim-methyl)	125+150+125	0.5

Appendix 11. Cultivation operations carried out on Equinox and Consort and Claire.

Equinox, 2002

Date	Operation/ substance	Common name of product	Active ingredient g l^{-1}/kg^{-1}	Applied field rate l or kg ha^{-1}
08.09.00	Cultivation	Treatments		
08.09.00	Sown	Equinox		165
04.11.02	Herbicide	Cordelia (isoproturon)	500	4
04.11.02	Herbicide	Ardent (diflufenican + trifluralin)	40 + 400	1
04.11.02	Insecticide	Permasect (permethrin)	230	0.25
04.11.02	Nutrient	Manganese sulphate ($MnSO_4$)	96	1
04.04.03	Fertiliser	Crop Master	28N:5P:5K:6SO_3	110:20:20:23
05.06.03	Fertiliser	Crop Master	28N:5P:5K:6SO_3	110:20:20:23
13.06.03	Herbicide	Ally (metsulfuron-methyl)	20% w/w	30
13.06.03	Herbicide	Starane 2 (fluroxypyr)	200	1
13.06.03	Nutrient	Manifol (Mn)	500	1
13.06.03	Fungicide	Landmark (epoxiconazole + kresoxim-methyl)	125+125	1

Claire and Consort, 2002

Date	Operation/ substance	Common name of product	Active ingredient g l^{-1}/kg^{-1}	Applied field rate l or kg ha^{-1}
	Ploughed Power harrowed			
18.10.02	Drilled	Consort and Claire		170
15.11.02	Herbicide	Ardent (diflufenican + trifluralin)	40 + 400	0.7
15.11.02	Herbicide	Trooper (flufenacef + pendimethalin)	60 + 300	2
15.11.02	Insecticide	Permasect (permethrin)	230	0.25
15.11.03	Nutrient	Manifol (Mn)	500	1
18.03.03	Nutrient	Manganese sulphate ($MnSO_4$)	96	3
18.03.03	Nutrient	Manifol (Mn)	500	0.5
18.03.03	Nutrient	Coptrel (Cu)	500	0.25
18.03.03	Fungicide	Folicur (tebuconazole)	250	1
04.04.03	Fertiliser	Crop Master	28N:5P:5K:6SO_3	110:20:20:23
15.05.03	Herbicide	Ally (metsulfuron-methyl)	20% w/w	30
15.05.03	Herbicide	Starane 2 (fluroxypyr)	200	1
15.05.03	Nutrient	Manifol (Mn)	500	1
15.05.03	Nutrient	Manganese sulphate ($MnSO_4$)	96	5